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CONTENTS

The Efficiency of Milk Substitutes in Calf Feeding. GEO. SPITZER AND R. H. CARR..... 315
The Water Buffalo as a Dairy Animal. C. O. LEVINE..... 340
The Relative Value of the Methylene Blue Reduction Test, the Bromthymol Blue Test, and the Bromcresol Purple Test in Determining the Keeping Quality of Milk. E. G. HASTINGS AND AUDREY DAVENPORT..... 353
The Digestibility of Sorghum Mill Refuse. ANDREW C. MCCANDLISH..... 367
The Digestibility of Corn Cannery Refuse. ANDREW C. MCCANDLISH..... 370
A Bacteriological and Biochemical Study of Experimental Butters. CHAS. W. BROWN, LULU M. SMITH AND G. L. A. RUEHLE..... 375
An Associative Study of Strept. lacticus and B. subtilis in Milk. MAX S. MARSHALL... 406
The Use of Fermented Milk and Milk Diets in Controlling Intestinal Putrefaction. R. C. FISHER 414
A Comparison of the Decantation Method with Other Methods for the Determination of Fat in Butter. O. R. OVERMAN AND SAICHI OKIMOTO..... 425
Abstracts and Reviews of Dairy Literature. H. A. HARDING..... 430
Dairy Notes. J. W. HENDRICKSON..... 431

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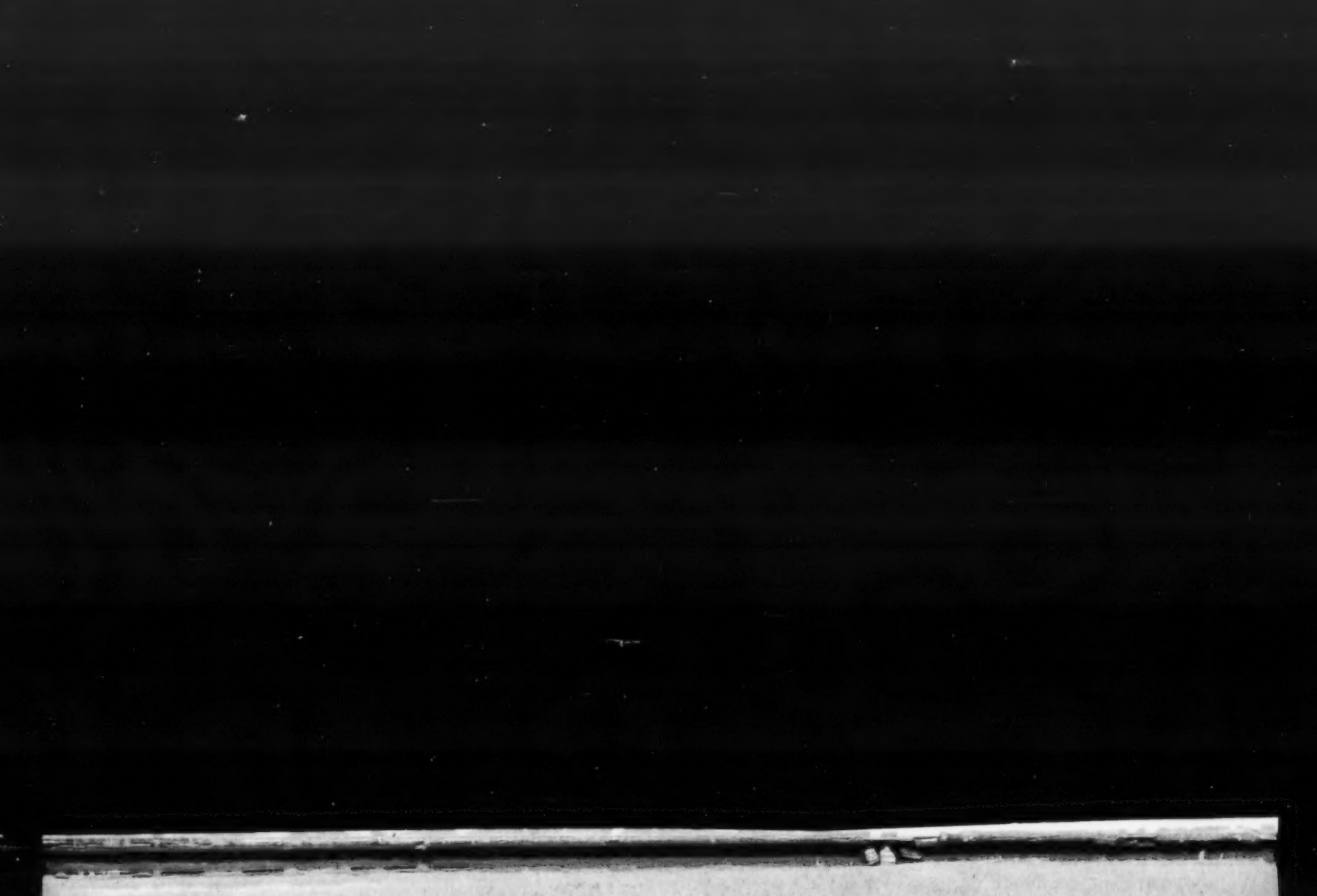

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THE EFFICIENCY OF MILK SUBSTITUTES IN CALF FEEDING[1]

GEO. SPITZER AND R. H. CARR
Dairy Husbandry Department, Purdue Experimental Station, Lafayette, Indiana

INTRODUCTION

For many years there has been a desire among dairymen to secure a milk substitute for the rearing of young calves. This desire and need have increased as the cost of milk production has increased. During our recent war the demand for whole milk increased greatly and consequently the price of milk and its by-products advanced to such an extent as to make the expense of raising calves on whole milk almost prohibitive. Although skim milk has proved to be an excellent substitute for whole milk after the calf has reached the age of two to three weeks, the supply of skim milk also is limited especially by dairymen who supply the customer with whole milk. There is no doubt but that whole or skim milk is the ideal food for the growing animal. The only difference between whole milk and skim milk is the fat content, so that in feeding skim milk the nutritive value of the fat must be supplied by other feeds such as corn, oats, etc. The grains supply the carbohydrates which serve as a source of energy. A rational feed to insure growth, maintenance and health must contain an ample quantity and quality of food materials, also a sufficient amount of both types of vitamines, the "fat soluble A" and "water soluble B," in addition the requisite amount of mineral matter.

RECENT INVESTIGATION

For many years nutritional investigators were of the opinion that the bodily growth and well-being of animals were dependent primarily on properly balanced rations and that such rations

[1] Acknowledgment is given Mr. R. E. Caldwell for his help during the first and second part of this experiment.

would meet the requirements and be considered complete foods if they consisted of certain definite proportions of any fats, proteins, carbohydrates and mineral salts. While in a large number of feeding experiments the growth obtained went far to prove the theory advanced, more recent investigations have shown conclusively that the above hypothesis is not valid, especially in regard to the proteins. Proteins are complex organic compounds built up of a large number of simpler definite chemical bodies called amino acids which have been designated by the term "Bau Steine" or building stones. It has been found that proteins derived from different sources vary considerably in quantity and kind of amino acids they yield. In some feeds certain amino acids are wanting, or if present are not in sufficient quantity. It is the kind and quantity of amino acids which give quality to the different proteins. Two amino acids play an especially important rôle in the growth and maintenance of animals. These are lysin and tryptophane. Therefore there are important differences in feeds resulting from the character of proteins derived from different sources. Numerous experiments have shown that there are great differences in the amounts of different proteins required to maintain animals without loss of body weight. It was found at the Wisconsin Experiment Station that there was a great difference in the value of proteins when derived from different sources. Experiments showed that the pig was able to retain for the construction of body tissue the following percentages of proteins in the naturally occurring food stuffs.

	per cent
Oil meal protein	16–17
Wheat[2] protein	20
Corn[3] protein	24
Oat[4] protein	25
Wheat germ protein	40
Skim milk protein	63
And a mixture of:	
Corn protein 90 per cent plus oil meal protein	10–31
Corn protein 75 per cent plus oil meal protein	25–37
Corn protein 60 per cent plus oil meal protein	40–31

[2] Hart, E. B., and McCollum, E. V., J. Biol. Chem., 1914, xix, 373.
[3] McCollum, Simmons and Pitz, J. Biol. Chem., 1916–1917, xxviii, 153.
[4] McCollum, Simmons and Pitz, J. Biol. Chem., 1917, xxix, 341.

This table shows that there is a great difference in proteins derived from different sources, and suggests the possibility of combining two or more groups of proteins which will be more efficient in promoting growth than the proteins in the individual grains. From these data and numerous other experiments the results of which are more or less relative, it is evident that to base the protein content irrespective of the character will lead to disappointing results in the feeding of animals.

VITAMINES OR FOOD ESSENTIAL "FAT SOLUBLE A" AND "WATER SOLUBLE B"

The most recent investigations have shown that in addition to a well balanced ration of fats, carbohydrates, proteins and salt of proper quality there must be present in a complete feed other nutritional essentials in order to promote growth, maintenance and well-being of animals. These substances are popularly designated by the term "vitamines." The term "vitamines" was suggested by Casimir Funk as one to designate a number of substances of unknown chemical composition to which he ascribed the property of preventing, curing or controlling diseases such as scurvy, pellagra, polyneuritis, etc. To a lack of vitamines in the diet these deficiency diseases were attributed. Researches by McCollum tend to establish the existence of two types of these food essentials. These he has designated as "fat soluble A" and "water soluble B." Vitamines occur in all plants and animals in varying amounts. The "water soluble B" is abundant in nearly all natural foods, but is almost entirely absent in purified sugar, starch, polished rice and fats of both vegetable and animal origin such as lard, tallow, olive oil, cottonseed oil, etc. The yolk of eggs, fats of internal organs, as the kidney and heart, are relatively rich in the "fat soluble A." Those foods containing relatively small amounts of the fat soluble accessory are cabbage, turnips, carrots and most other root crops, and all highly sterilized products.

The leafy portion of growing plants is relatively rich in vitamines, differing markedly from the seeds. Rations of seeds,

tubers, and roots, although supplemented with the required mineral salts, are insufficient to maintain life, but the addition of the leafy parts of plants to this combination will furnish a food which is adequate to support life.

Milk, the best of natural foods, is rich in all essentials for the maintenance, growth and well-being of animals. It includes large amounts of both the fat soluble and water soluble vitamines.

MINERAL REQUIREMENTS OF THE ANIMAL

The growth of an animal involves the utilization of mineral matter in the formation of tissue, as well as of the skeleton. The greatest need is for bone formation. About two-thirds of the dry matter of bone consists of mineral matter. So it will be seen readily that it is very important that there should be a sufficient quantity of the mineral elements for the growing animals. These mineral elements are derived from the feed; and inasmuch as some feeds, especially grains, are very deficient in either one or more of the essential salts, it is highly essential to supply this deficiency. The more restricted the ration as to variety of feeds, the greater the danger of an insufficient supply of the proper mineral elements. Those most frequently deficient in feeds are sodium, calcium, and phosphates.

CALF FEEDING EXPERIMENTS

In 1915–1916 O. F. Hunziker and R. E. Caldwell conducted experiments in skim milk and milk substitutes for calf feeding. (Bulletin No. 193, this Station). Thirty calves of pure-bred stock were divided into lots of ten calves each. One lot was fed *skim milk*, another was fed *home mixed meal* and a third lot was fed Blatchford's Calf Meal. The following year, 1916–1917, O. H. Anderson, in coöperation with the authors, conducted a more extensive experiment on the efficiency of certain milk substitutes in calf feeding. These results were published in the Journal of Biological Chemistry, vol. xxviii, 1917. The object of this investigation was; first, to determine the growth efficiency of certain milk substitutes in calf feeding; second, to determine

the nitrogen distribution; third, to determine the nitrogen utilization of the different feeds used.

The following combinations were used in this experiment:

I. Vegetable meal. Linseed meal, soy bean meal, cottonseed meal and wheat middlings—equal parts by weight of each feed.

II. Home mixed meal. Hominy feed, linseed meal, cottonseed meal, White Swan flour, dried blood, equal parts by weight of each feed.

III. Vegetable dried blood. Soy bean meal, linseed meal, cottonseed meal, wheat middlings, and dried blood, equal parts by weight of each feed.

IV. Home mixed casein meal. Hominy feed, linseed meal, White Swan flour and casein, equal parts by weight of each feed.

From the results obtained by these preliminary experiments, especially from the work done by O. H. Anderson and others, it seemed advisable to continue this feeding experiment. The last experiment showed clearly that there was a great difference in the structure of the proteins as shown by the nitrogen retained for body growth and the distribution of the excreted nitrogen in the feces and urine.

PURPOSE OF EXTENDED EXPERIMENTS

The object of these experiments was to find a milk substitute which would be more efficient for the rearing of calves than the feeds already tried for this purpose. In order to determine the efficiency of any calf feed its success must be compared with that of milk as a standard. The experiment involved the comparison of the calves fed substitutes with those fed milk as to their growth and health, utilization and digestion of feeds, and the distribution of nitrogen in the urine and feces.

EXPERIMENT I

The investigators wished to test particularly the efficiency of liquid beef blood as compared with milk and also that of purely vegetable proteins. Analyses were made of all feeds or mixtures of feeds and an accurate record kept as to the amount fed. Total

nitrogen was determined in urine and feces of the daily composite. Feeding crates made it possible to collect urine and feces separately and keep a quantitative daily record of the nitrogen excreted.

Method of procedure

The calves used in this experiment were grade Holstein, weighing 85.8 to 113.9 pounds and from six to nineteen days old. They were selected with respect to their uniformity in vigor and general condition. During the experiment period the calves were fed in crates. They were given frequent exercise on the barn floor and weighed every ten days. At the beginning they were fed on skim milk for a period of six to ten days. Then the milk substitutes were used as a part of the feed and gradually increased in such quantities as seemed compatible with the maintenance of the natural functional operations. At the end of ten days the calves that received milk substitutes were no longer fed any milk.

Feeding chart for experiment I. Feeding periods and feeds received by each calf

	CALF 22	CALF 110	CALF 219	CALF 223
Period I	Liquid blood Hominy Linseed meal Red Dog flour	Skim milk	Skim milk	Clover infusion Corn meal Gluten meal Red Dog flour Buckwheat flour
	Alfalfa hay	Alfalfa hay	Alfalfa hay	Clover hay
	Dry mash Ground oats and corn	Dry mash Ground oats and corn	Dry mash Ground oats and corn	Dry mash Corn gluten Corn meal Buckwheat, ground Red Dog flour

Discussion of experiment I

In planning this feeding experiment it was the aim to select a simple combination of grains which are available and can be purchased at a reasonable price. In this experiment a comparison has been made between the efficiency of the proteins derived from liquid blood in combination with vegetable proteins, the liquid blood supplying the animal proteins, and that of feeds composed entirely of vegetable proteins. The liquid beef blood was used in place of dried blood which is used considerably as a source of protein.

In drying blood, the proteins become insoluble in water, and owing to the high temperature to which the blood is subjected in drying and sterilization, it seems probable that some of its nutritional value might be destroyed. Liquid blood supplies the amino acids which, to a great extent, are required to synthesize the proteins of the animal. Besides, in the liquid blood the proteins are in their natural condition, and contain the food accessories in their active state.

Our feeding experiment bears out the superiority of liquid blood to dried blood in effecting metabolism, the physiological process by which non-living matter is built up into living matter or living matter broken down into more stable compounds. This difference is strikingly shown in the behavior of calf 22. When the calf was fed on the liquid blood ration, the nitrogen eliminated in the urine was 45.81 per cent of the nitrogen intake, while with the calves fed on vegetable proteins plus clover infusion and also milk, the nitrogen eliminated in the urine was 27.33 per cent of the nitrogen intake, and in the milk fed calves the nitrogen in the urine was 31.2 per cent. From our previous feeding experiment, it would seem that dried blood in combination with vegetable proteins had no more effect in stimulating metabolism than the feed composed entirely of vegetable proteins.

While it is realized that liquid blood is not practical as such, yet this experiment furnishes evidence of its superiority as a feed over dried blood and it is possible to prepare blood in a

dry and at the same time more soluble form, which, to a great extent, will have the nutritive value of liquid blood. In the feed composed of vegetable proteins the basic feed was similar in character of the proteins to that fed in the liquid blood mixture. The liquid used in the feed for calf 223 was an infusion of clover hay which in some communities is being used to supplement milk. The dry mash and roughage were also of similar character in the two lots. The clover hay infusion was heated to boiling and mixed with the requisite amount of dry basic feed. This food was prepared daily. Inasmuch as the ration of the two lots of calves consisted of grains of low feeding value and was deficient in both the unidentified dietary factors, "fat soluble A" and "water soluble B," and since the leaves contain the dietary factors in much greater quantity than the seeds, the aim was to supply these by the use of clover hay infusion. This experiment with the clover infusion was conducted primarily for the purpose to test the efficiency of purely vegetable proteins.

In feeding the calves, sufficient quantities of the mixtures were fed to keep the animals in normal condition, the physical condition of the feces being used as criterion. Dry mash and roughage were supplied in sufficient quantity to satisfy the wants of the animals.

Summary of experiment I

I. The following represents the average daily gain in pounds of the three representative feeds, liquid blood, clover infusion, and milk, during the first ninety days, or the critical period of the life of the calf.

	pounds
Calf 22: Liquid blood ration	1.32
Calf 219: Milk ration	1.40
Calf 110: Milk ration	1.17
Calf 223: Clover infusion ration	1.03

II. The following per cent of nitrogen fed was retained.

	per cent
Liquid blood ration	24.4
Milk	42.8
Clover infusion ration	31.9

III. Of the nitrogen intake, the excreted nitrogen was distributed as follows:

	per cent
Liquid blood ration:	
In urine	45.9
In feces	29.9
Milk ration:	
In urine	31.2
In feces	26.7
Clover infusion ration:	
In urine	27.4
In feces	40.7

IV. The average daily nitrogen intake for the calves was as follows:

	grams
Calf 22	85.96
Calf 110	57.41
Calf 219	38.66
Calf 223	48.72

V. Average daily gain in body weight per gram of nitrogen retained.

Calf 22	28.5
Calf 219	38.4
Calf 110	22.8
Calf 223	30.2

EXPERIMENT II

Introduction

From our previous experiment[5] in feeding liquid beef blood as a source of animal protein, we deemed it worth while to continue the use of blood protein as a substitute for milk protein. As stated in our previous discussion and as must be evident to any feeder, liquid blood, as used in our experiment, is not a practical feed, owing to the difficulty of securing a sufficient quantity at regular intervals. This would be possible only in locations where large slaughter houses are located. The greatest objectionable feature in the use of liquid blood is its rapid deterioration, which makes it unfit for this purpose.

[5] Caldwell, R. E., Dairy Science, ii, 312.

To secure what seemed to us a practical combination, judging from results obtained from our previous feeding experiments with mixtures of grains and proteins from different sources, we selected for our mixture, ground corn, oil meal, and liquid beef blood. The proportions were corn meal, 8 parts, oil meal 1 part, and liquid blood 12 parts, corresponding approximately to 3 parts of dried blood. The proportion of corn meal and oil meal were suggested by work done at the Wisconsin Agricultural Experiment Station. It was found that when corn alone was fed the proteins were utilized to the extent of 24 per cent and oil meal alone 20 per cent, but when mixed in the proportion of corn protein 75 per cent and oil meal protein 25 per cent and fed, 40 per cent of these proteins was utilized in the formation of body protein. These grains are easily available and moderate in price, and the mucilageous body in the oil meal serves an excellent purpose in keeping the mixture in suspension when mixed with warm water.

Purpose

The purpose of this experiment was to determine the efficiency of this mixture of corn meal, oil meal, and liquid blood when prepared in a dry condition, which ultimately could be manufactured on a large scale and made a practical commercial feed for raising young calves.

Preparation of feed

In preparing the mixture of corn meal, oil meal, and liquid blood for this feeding experiment particular attention was given to the drying. It is generally accepted that high heat, at the boiling point of water deteriorates or has a destructive influence on the activity of the unknown dietary factors, "vitamines." And no doubt, continued high heat, especially above the boiling point of water, causes the proteins to become less soluble and digestible in the alimentary tract. In order to avoid the effect of high heat, the mixture was dried on shallow tin pans at a temperature not above 140° to 160°F. The mixture was stirred

frequently. During the drying a warm blast of air was constantly forced over the mixture, this procedure hastening the drying and avoiding undue exposure. This drying was completed in 4 to 6 hours sufficiently to permit grinding. After drying the feed was ground fine enough to pass a forty mesh sieve. This feed, when mixed with water 160° to 180°F. and left standing for a short time, becomes gelatinous and can be diluted with water to the required consistency for feeding. The mixture of corn meal, oil meal and liquid blood as prepared above, we will designate in the future as "Purdue Calf Meal."

Method of procedure

Two Holstein grade male calves of about the same age were selected for this feeding trial. The calves weighed 119 to 129 pounds, age fourteen to sixteen days respectively. They were fed in feeding crates as in our previous experiment. Milk having been fed for two days it was replaced by the calf meal. This was gradually increased daily so that at the end of seven days the calves were fed exclusively on this meal. In addition to this prepared feed the calves were fed a dry mash consisting of ground corn 8 parts, and oil meal 1 part, in such quantity that the trough was clean of mash at each feeding. The calves had access to roughage of chopped corn fodder of excellent grade, of which they were allowed all they would eat. The uneaten portion, consisting of some husks and stocks was removed daily and fresh fodder supplied. A record was kept of all feeds fed and consumed.

Nitrogen content of feeds

	per cent N
Milk	0.5
Blood mixture	4.24
Dry mash	1.98
Stover	1.00

Discussion of experiment II

In this discussion it is our aim to show the efficiency of Purdue Calf Meal compared with that of liquid blood and milk.

The average daily gain of calf 22, fed on liquid blood, was 1.32 pounds, calves 219 and 110, receiving milk, made an average daily gain of 1.40 and 1.17 pounds respectively, while calves 1 and 2 receiving Purdue Calf Meal made an average daily gain of 0.96 and 0.94 pounds.

From the summary it is seen that the average daily nitrogen intake for calf 22 was 85.96 grams, and for calves 219 and 110 the average daily nitrogen intake was 38.66 and 57.47 grams respectively. If we consider the nitrogen retained for body growth of the different rations, we find that calf 22 retained 21.09 grams of nitrogen daily, calves, 219 and 110 retained 16.6 and 23.25 grams daily while calves 1 and 2 retained 19.58 and 21.81 grams of nitrogen daily. From these figures it appears that calf 22 received more nitrogen than the capacity for growth required. When the digestible nitrogen intake is greater than that required for growth, the excess is catabolized (that is, the living protoplasm breaks down complex substances into simpler components), the residual nitrogen appearing in the urine. This seems quite evident from the figures obtained in the distribution of the excreted nitrogen. Summary I and II. By comparing the gain in weight per gram of nitrogen intake we find a close relation with the exception of calf 219.

Daily gain per gram of nitrogen retained

Calf 22	28.5
Calf 219	38.4
Calf 110	22.8
Calf 1	22.2
Calf 2	19.6

The figures show the nitrogen distribution of the calves fed different rations.

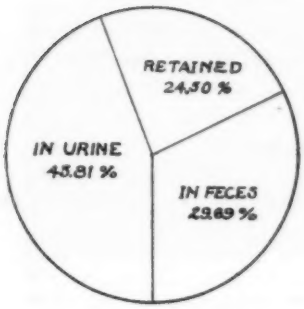


FIG. 1

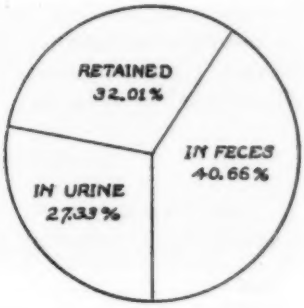


FIG. 3

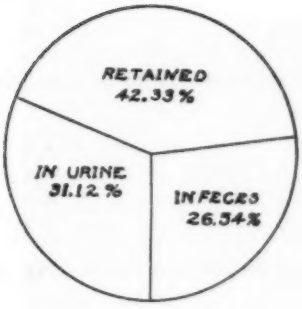


FIG. 2

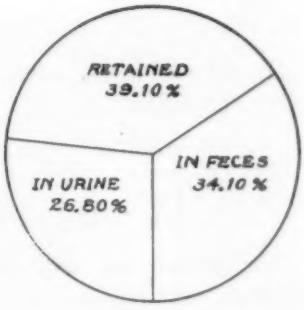


FIG. 4.

Figure	*No.*	
1	22	Liquid blood ration
2	110, 219	Milk ration (average)
3	223	Clover infusion ration
4	1, 2	Purdue Calf Meal ration (average)

The circles represent the total nitrogen intake, and each sector shows nitrogen retained, excreted in feces and urine.

These figures together with the gain in weight per gram of nitrogen intake represent the efficiency of Purdue Calf Meal as a feed for young calves.

Summary of experiment II

I. The following is the average daily gain in pounds for the two calves fed Purdue Calf Meal.

	pounds
Calf 1	0.96
Calf 2	0.94

II. The following per cent of nitrogen fed was retained.

	per cent
Calf 1	37.03
Calf 2	41.20

III. Of the intake nitrogen the excreted nitrogen was distributed as follows:

	per cent
Calf 1, in urine	27.5
Calf 1, in feces	35.5
Calf 2, in urine	26.1
Calf 2, in feces	32.7

IV. The average daily nitrogen intake for the calves was as follows:

	grams
Calf 1	52.92
Calf 2	52.94

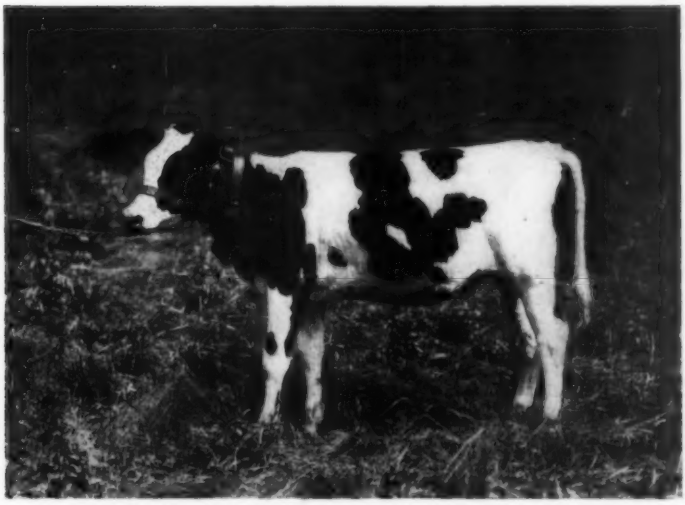
V. Average daily gain in body weight per gram of nitrogen intake.

Calf 1	8.2
Calf 2	8.1

VI. Average daily gain in body weight per gram of nitrogen retained.

Calf 1	22.2
Calf 2	19.6

A Group of Calves Fed Purdue Calf Meal



One of The Experimental Calves Fed Purdue Calf Meal,
Age 5 Months

EXPERIMENT III[6]

Introduction

To follow on our experiments for the past several years in feeding young calves in crates different mixtures of feeds, and because of the comparatively uniform results of the feeding of blood meal, we were led to try this prepared calf feed with a large number of calves. It is evident that crate feeding can not give the same results as when calves are fed in stalls or when conditions are more favorable for development and well-being of animals. In crate feeding the calves are deprived of two of the essentials of life, exercise and freedom. In order to test the efficiency of any mixed feed and draw conclusions as to its relative value, if it is to become a commercial product, it must be fed to a larger number of animals having more natural environments, such as out door exercise and pasture.

Purpose

From the practical feeder's point of view, it seemed highly desirable to know the development and well-being of the animals of this experimental feeding after the earlier stages or critical period of the young animal's life. To test the efficiency of this feed after the first seventy to seventy-four days' feeding, the feeding was continued seventy to seventy-seven days longer, making a total of one hundred and forty to one hundred and fifty-one days. To compare the efficiency of Purdue Calf Meal with that of other feeds, two lots of calves of approximately like age, weight and general condition were fed on milk, one lot on skim milk and one lot on whole milk. It was also our purpose to test the performance of the three lots of calves after the expiration of the second feeding period when they were put on blue grass pasture. The three lots of calves during this

[6] Acknowledgment is due to Prof. O. E. Reed, chief of the dairy department, for his interest in these experiments and for the extra means and privileges which made the third feeding experiment possible. Also to Swift and Company of Chicago, Illinois, for their kindness in preparing the calf meal especially for us as used in our last feeding experiment.

period had the same natural advantages. The pasturing was to be continued until the calves were one year old.

Preparation of feed

Owing to the larger number of calves fed in this experiment and the limited facilities in the laboratory, the calf meal was manufactured by Swift and Company in quantities sufficient to complete the experiment. The same proportions of corn meal 8 parts, oil meal 1 part and liquid blood 12 parts were used; in addition 1 per cent of bone meal was added, the drying was done at a temperature as near as possible to that of our previous feeding experiment, when the calf meal was made in the laboratory. After drying, the meal was ground to pass a 30 to40 mesh sieve.

Number of calves used in the following experimental feeding

Thirty-six grade Holstein calves were purchased from the stock yards at Chicago for use in this experiment. These calves ranged in age from ten to twenty days as far as could be determined. They were remarkably uniform in vigor and general appearance, weighing from 85 to 126 pounds. The thirty-six calves were divided into three lots. Lot I and II received whole milk and skim milk respectively, lot III received Purdue Calf Meal. The total weight of each lot of 12 calves was: Lot I, 1205; lot II, 1180; and lot III, 1201 pounds.

Feeding and stabling

Feeding. Each calf was taught to drink from a pail on arriving from the stock yards. They were all fed on whole milk for ten days. Lot II was gradually changed to skim milk. Lot III was changed to Purdue Calf Meal by gradually increasing the quantity of Calf Meal. Ten days were taken to make the change from whole milk to skim milk and calf meal. Lots I and II fed on whole milk and skim milk, received milk in proportion to their live weight, 10 pounds of milk daily per 100 pounds weight.

This ratio was continued until calves weighed 200 pounds and were receiving 20 pounds of milk. No increase in the quantity of milk fed was made after the calf weighed 200 pounds. Lot III fed on calf meal, received a definite quantity of meal per day based on proportionate amount of nitrogen contained in milk and this was continued throughout the feeding period. All calves were fed a grain ration composed of equal parts of ground corn and oats. The ground grain was weighed for each lot of calves daily and a record kept of the amount eaten. The calves had access to good clover hay which was supplied in sufficient quantity to meet their wants and was not wasted. They were watered at regular intervals.

Method of feeding calf meal

Several preliminary trials were made concerning the quantity of calf meal which a young calf could eat without causing digestive disturbance, scours and consequent failure to eat.

The first trial of feeding the required amount to a lot of calves was based on the nitrogen content of the milk fed. Knowing the percentage of nitrogen in milk and that of the feed, a nitrogen equivalent of the feed can be calculated corresponding to the quantity of milk. In our first trial the digestibility of milk and meal was taken into consideration, and a proportionately large quantity of meal was fed. We soon realized that the calves were over-fed. They were all subject to scours and indigestion which was manifested by the condition of the feces and their refusal to eat. On reducing the quantity fed to a much smaller amount they soon regained their normal condition. In our next trial of feeding the meal the amount fed was based on the nitrogen equivalent of milk.

The calculations were based on the percentage of nitrogen in milk at 0.5 per cent and that nitrogen of Purdue Calf Meal at 5.0 per cent. The milk-fed calves received 10 pounds of milk daily for each hundred pounds of live weight or 120 pounds of milk for twelve calves weighing 100 pounds each (or per lot of total weight of 1200 pounds live weight).

120.0 pounds of milk (N. O. 5 per cent) = 280 grams of nitrogen
12.1 pounds of Purdue Calf Meal = 275 grams of nitrogen

This proportion of calf meal was fed with a *decided* improvement. For very young calves it was found to be too much, causing scouring among the weaker calves but no apparent indigestion was shown by the appearance of the feces.

Another feeding trial was made, reducing the quantity of the meal so that the nitrogen from this source was 80 per cent of that contained in the milk. Feeding this proportion of calf meal, that is 9.6 pounds of meal to 120 pounds of milk proved to be the safe and correct amount to feed.

This quantity, 9.6 pounds of calf meal to 1200 pounds of live weight, was fed to lot III during the feeding period. We believe this proportion can be safely increased to 90 per cent of that of milk nitrogen after the calf has reached the age of ninety or one hundred days. Much will depend on the breed and individual vigor of the calves. The feeder must be the final judge. A safe criterion concerning the feeding is the physical condition of the feces when compared with the feces of a normal milk-fed calf. The above quantity, 9.6 pounds of calf meal, is the maximum quantity which we were able to feed during the feeding period.

Preparation of feed and quantity of calf meal used for feeding

If the total weight of a lot of calves is known, it is a simple matter to calculate the amount of meal required for the lot. To feed each calf its proper proportion based on its weight, the weight of each calf must be known. To prepare the calf meal for feeding, the meal is weighed and placed into a container of sufficient capacity to hold the mixture when properly diluted. To the meal in the container about four times its weight of warm water at 160° to 180°F. is added and well mixed. This is left standing for one to two hours and then diluted to a consistency for the calves to drink. The feed is prepared twice daily and fed at blood heat, about 98° to 99°F.

When feeding a lot of calves the diluted calf meal, the weight of each calf must be taken into consideration to properly apportion the feed on the basis of live weight. It is convenient to dilute the mixture to a total weight which is divisible by the number of calves. In our feeding where 12 calves were fed, the lot weighing 1200 pounds, 4.8 pounds of calf meal was prepared as previously directed and diluted to 48 pounds per feed. This would require 4 pounds of the diluted feed to each 100 pounds of live weight.

Table showing the quantity of the liquid prepared feed fed to a lot of 12 calves

NUMBER OF CALF	WEIGHT OF CALF	POUNDS OF PREPARED FEED AT EACH FEED
1	85	$0.85 \times 4 = 3.40$
2	88	$0.88 \times 4 = 3.52$
3	126	$1.26 \times 4 = 5.04$
4	93	$0.93 \times 4 = 3.72$
5	96	$0.96 \times 4 = 3.84$
6	123	$1.23 \times 4 = 4.92$
7	100	$1.00 \times 4 = 4.00$
8	100	$1.00 \times 4 = 4.00$
9	114	$1.14 \times 4 = 4.56$
10	95	$0.95 \times 4 = 3.80$
11	87	$0.87 \times 4 = 3.48$
12	94	$0.94 \times 4 = 3.76$

Total weight, 1201; weight of feed fed, 48.04.

When the total weight of the lot of calves reaches 1400 pounds it would require 0.8 pound more of the meal per feed for the twelve calves and the liquid mixture made up to 56 pounds. Again multiply the weight of each calf at this weighing, expressed in hundredths, by 4; this will give the required amount of liquid feed to feed each calf. The above mentioned dilution and proportioning has proved successful in our feeding experiments. It must not be inferred that the rate of dilution must be strictly adhered to. Each feeder must be guided by the behavior of the calves; for some calves, or at different periods of feeding, the consistency of the liquid calf feed would require to be changed.

Weighing

The calves of these lots were weighed at intervals of seven days. The weighings were made at about the same time each weigh day. This was always done in the forenoon after the morning feeding.

Pasture

All calves had access to blue grass pasture in season when weather permitted them to be turned out. They also had access to clover hay when out in the open. The hay was placed in an open rack sufficiently large to accommodate all calves without crowding.

Discussion of experiment III

In this experiment we endeavored to show the efficiency of Purdue Calf Meal when fed to a larger number of calves, eliminating individual peculiarities to a much greater extent. In the absence of a chemical analysis of the excreta only a comparison of the nitrogen intake and gain in body weight can be made which was deemed sufficient for the purpose of this experiment.

In summary III it will be seen that the average daily nitrogen intake for the three lots of calves was rather uniform. During the first feeding period the grams of nitrogen intake were for lots I, II, III, 52.1, 54.7 and 52.5.[7] For the second period the nitrogen intake was for lots I, II and III, 85.4, 90.3, and 94.3 grams daily.

If we compare the gain in body weight per gram of nitrogen intake from the different sources, we find the average daily gain in weight for the entire feeding period to be as follows:

	Grams daily gain
Whole milk ration	13.5
Skim milk ration	11.0
Purdue Calf Meal	7.3

[7] By nitrogen intake we mean the nitrogen derived from the milk, calf meal and mash. Clover hay was fed to all lots, but no record kept and may be considered a constant.

It will be noted that the skim milk and calf meal ration produced a gain in weight per gram of nitrogen in this experiment approximately the same as in our previous experiments.

During this feeding experiment the calves fed Purdue Calf Meal received less than 80 per cent as much nitrogen from the calf meal as the milk fed calves received from milk. The balance of the nitrogen intake was derived from the mash. The effect of the nitrogen derived from mash in the growth of the calf is shown by comparing the gain in weight of the three lots during the first and second period. During the earlier stages of feeding, the proteins derived from the mash were not utilized for growth as during the second period of feeding.

The three lots of calves, fed whole milk, skim milk, and calf meal received the following average daily quantity of nitrogen from these sources.

Period I

Lot I	*Lot II*	*Lot III*
34.9 grams of nitrogen	35.5 grams of nitrogen	19.3 grams of nitrogen

Period II

45.0 grams of nitrogen	46.1 grams of nitrogen	35.2 grams of nitrogen

If we base the gain in body weight on the nitrogen obtained from whole milk, skim milk and calf meal, we find a close relation of gain in weight for the three lots of calves.

Average daily gain in weight per gram of nitrogen

Period I

Lot I	*Lot II*	*Lot III*
Whole milk, 22.3 grams	Skim milk, 18.0 grams	Calf meal, 19.4 grams

Period II

Whole milk, 22.9 grams	Skim milk, 20.7 grams	Calf meal, 20.0 grams

From the above figures it seems that in all Lots the proteins derived from animal origin, that is, milk and blood, are the principal factors in promoting growth and approximately equal in efficiency gram for gram. The nitrogen derived from the mash must have been utilized to a certain extent in the production of growth but not in proportion to the quantity consumed.

The energy required to digest proteins and carbohydrates from grains must be taken into consideration in feeding young calves. This is an important factor especially where the energy required to transform the food into an assimilative condition may nearly equal that required for maintenance. This is shown in feeding of the calf meal during the first and second periods. During the first period of 70 days the calves made an average daily gain of only .83 pounds, and in the second period an average daily gain of 1.53 pounds. This emphasizes the ability of the calves to utilize the feed much more efficiently in the second period after the calves reached the age of eighty to ninety days than in the first period. This was further emphasized during pasturing after the experimental feeding, when the calves from the three lots made the same average daily gain during the six months when on pasture.

Summary of experiment III

The following represents the average daily gain in weight for the three feeds, whole milk, skim milk and Purdue Calf Meal.

I. Gain during first period:

	pounds
Whole milk ration (Lot I)	1.72
Skim milk ration (Lot II)	1.41
Purdue Calf Meal ration (Lot III)	0.83

II. Gain during second period:

	pounds
Whole milk ration (Lot I)	2.26
Skim milk ration (Lot II)	2.06
Purdue Calf Meal ration (Lot III)	1.53

III. Average daily grams of nitrogen consumed during first feeding period:

	grams
Whole milk ration (Lot I)	52.1
Skim milk ration (Lot II)	54.7
Purdue Calf Meal ration (Lot III)	52.5

IV. Average daily grams of nitrogen consumed during second feeding period:

	grams
Whole milk ration (Lot I)	85.4
Skim milk ration (Lot II)	90.3
Purdue Calf Meal ration (Lot III)	94.6

V. Average daily gain in body weight per gram of nitrogen consumed:

First period

	grams
Whole milk ration (Lot I)	14.9
Skim milk ration (Lot II)	11.7
Purdue Calf Meal ration (Lot III)	7.2

VI. Average daily gain in body weight per gram of nitrogen consumed:

Second period

	grams
Whole milk ration (Lot I)	12.1
Skim milk ration (Lot II)	10.3
Purdue Calf Meal ration (Lot III)	7.4

VII. Average daily gain in body weight for the entire feeding:

	pounds	*days*
Whole milk ration	1.91	140
Skim milk ration	1.73	140
Purdue Calf Meal ration	1.18	151

VIII. Gain in weight of the different lots when put on pasture: After the 140 days feeding, the calves were put on blue grass pasture, and during the six months the following average daily gains were made by each calf of the different lots.

	pounds
Whole milk ration (Lot I)	1.16
Skim milk ration (Lot II)	1.24
Purdue Calf Meal ration (Lot III)	1.18

IX. At the age of eighteen months the average weight per calf was for:

	pounds
Whole milk ration (Lot I)	582
Skim milk ration (Lot II)	594
Purdue Calf Meal ration (Lot III)	475

The difference in weight of the calves fed calf meal and milk occurred principally during the first period of seventy to ninety days.

SUMMARY

1. Beef blood when combined with corn and oil meal in proportion of 8 parts corn meal, 1 part oil meal and 12 parts beef blood furnishes a reasonably successful milk substitute for feeding young calves.

2. The development of the calves when on pasture indicates that the feeding of Purdue Calf meal seems not to have impaired or stunted the growth impetus, as the gain in weight of calves fed Purdue Calf Meal was equal to the gain in weight of the milk fed calves while on pasture.

THE WATER BUFFALO AS A DAIRY ANIMAL

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HISTORY OF THE WATER BUFFALO

The original home of the water buffalo (Bubalus bubalis Lyd.), is not definitely known. However, credit for the first domestication of this class of bovine is probably due to the people of northern India where today the undomesticated form still exists, although in small numbers.

At an early date, the water buffalo was domesticated and had reached what is now southern and southwestern China. Chinese literature refers to it as having been used as a draft animal and as a source of beef for centuries by the farmers of southern China.

DISTRIBUTION TODAY

Today the water buffalo, as a domesticated animal, is common in practically all of India, Siam, Straits Settlements, Indo-China, China as far north as two hundred miles north of Shanghai, the Philippine and Hawaiian Islands, and other islands of the Pacific Ocean. From the Hawaiian group, they jumped across to Brazil in South America. At an early date, as early as 600 A.D., according to some writers, they reached southern Europe, where today, in Italy, they may be found plowing the small fields for the Neapolitan farmers. No attempt has ever been made to introduce them to the United States. Great numbers are kept on the bottom land of the Danube, the Theiss, and the Drave in Austria-Hungary, and in Italy on the plains lying to the north and east of Naples. Indian dairy breeds have been introduced to Trinidad, where they are used as draft animals and are both worked and milked by Hindu coolies.

Wild buffaloes are still to be found in India, in the grassy jungles along the large rivers of that land, and in the open marshy prairies, the finest specimens being in Assam and Burma, and in the Straits Settlements, Java, Ceylon, Sumatra and Borneo (1). A wild form, different from the Asiatic species, exists in parts of Africa. The African species is exceedingly dangerous and has never been tamed.

GENERAL DESCRIPTION OF THE WATER BUFFALO

When compared in size with other classes of animals in the bovine group, the water buffalo is a relatively large animal. The average size for mature animals of the beef-draft type, which is the type common in China and in the Islands of the Pacific, is between 800 and 1400 pounds. In height, they vary from 46 to 50 inches at the withers. The dairy breeds of northern and central India, especially those in the region of Delhi in the Bombay presidency, is much larger in size (2). A herd of thirty cows of this breed in Kowloon, near Victoria, Hongkong, which were studied by the writer in 1918, varied in height from 56 to 60 inches at the withers. The weight of this breed is considerably more than that of the beef-draft type common in China. Animals in ordinary flesh, in which condition there is no superfluous fat on the body, weigh from 1500 to 2000 pounds. Other minor dairy breeds of India are the Jafferabad, from the west, and Deccani and Nagpur breeds from the central part of India (2).

In color, the water buffalo is a dark grey, or almost black, with now and then white markings on their face and legs. Pure white albinos are now and then seen, especially in parts of Tonkin province in southwestern China, where they are in special favor because of their supposed immunity to rinderpest.

The skin of the water buffalo, like that of the pig, has practically no sweat glands, and, for this reason, the buffalo likes to wallow in mud or water on a hot day, and thereby it gets its name, "water buffalo." There are also apparently no oil glands in the skin of the buffalo. The mature animal has very little hair on its body. The sides, back, and thighs of the animal are

practically bare. The shoulders and knees have the most hair, where it is about 2 to 3 inches long, and very thin. The buffalo calves are born with a rather heavy coat of long, brown hair all over the body, which gradually disappears after one year of age is reached. The horns of the buffalo are characteristic, although they vary in size and shape, with different breeds. In general, those of the beef-draft type are long, thick and somewhat triangular at the base, fairly sharp at the tip, with distinct grooves on the upper surface. They are directed out, back and somewhat upward from the head, and curve inward at the tips. The length of the horns, measured on the outside of the curve, is usually about 2 feet, although they may vary from 12 inches to 4 feet or more. The movements of the water buffalo are rather slow and unwieldy, similar to that of the elephant; in fact, a casual view of a moving herd of buffalos suggests a roving band of elephants.

The udders of both the beef-draft types of China and dairy breeds of India are rather small, but well shaped, with well developed teats. The beef-draft animals have a wide back and loin, with short legs and a large barrel. The body, as a rule, is short and compact, and well-built for draft purposes. The croup is decidedly sloping. They fatten readily, and buffalo beef from young animals is similar to that of the Bos taurus or Bos Indicus, although it is darker in color.

There are a number of striking differences in the Delhi dairy breed of buffalo from India, and the beef-draft type mentioned above. The horns of the Delhi breed rise from the head in an upward and backward direction, and then curl over the head like the horns of a ram, and, for this reason, in the Philippine Islands they are called "ram's horn" buffalos. The body is lean and angular. The teats are well placed and of rather large size, usually somewhat constricted at their attachment to the udder. They have a very large frontal bone in the head, and small, wall eyes. Although rather fierce looking on account of their large horns, prominent forehead and glassy eyes, the Delhi buffalo are very docile and easily managed, by Europeans as well as by Indians. The beef-draft type of southern China, on the

other hand, while easily managed by the natives of that land, dislike strangers, especially Europeans, who find it difficult to manage.

The gestation period of the water buffalo varies from ten to eleven months. The calves generally weigh from 60 to 80 pounds at birth.

USES OF THE WATER BUFFALO

The principal use of the water buffalo in China and the Pacific Islands is for draft purposes. Surplus animals, or animals too old for good use in the fields are fattened and slaughtered for beef.

They possess great strength, although they are not fast in getting over the ground when compared with draft horses. It was found by Gandencio Evaristo (3) at Los Banos, Philippine Islands, that, in fairly light soil, and on a rainy or cloudy day, a buffalo could plow 5000 to 7000 square meters a day.

For uplands, where water is not available close at hand, the buffalo is of little use, except where work is limited to the early morning or late afternoon. This is because it is unable to stand the heat of midday without being kept constantly cooled with water on its body surface.

In India, where the religion of the natives largely prohibits the use of the flesh of animals for food, the buffalo is used for draft and dairy purposes only. When used for draft purposes, they are used singly, single-tandem, or two are yoked together and driven in the way which oxen are generally used. As a rule, in China only one buffalo is used on a plow. The plows cut a furrow slice from six to eight inches wide and two to five deep.

THE CHINESE DRAFT WATER BUFFALO AS A POSSIBLE DAIRY ANIMAL

No dairy breed of water buffalos has yet been developed in China. This is probably largely due to the fact that, until the advent of the Europeans in China, the Chinese did not consider

milk from cattle fit for human food. It is strange that this opinion should have so generally prevailed, for the Tabetans on the west and the Mongolians on the north have, from time immemorial, been users of dairy products in the form of milk, butter and cheese, made from cow's, goat's, yack's or mare's milk. It is only during the present generation that the Chinese have begun to use milk to any extent, and its use today is confined chiefly to the regions where the Chinese have come in contact with Europeans and western methods.

The fact that the milk of the Chinese buffalo cow is very rich in fat, and palatable, has been known in a general way for many years, but it is only some fifteen years ago that the first water buffalo dairy was started in Canton.

STUDY OF THE WATER BUFFALO AT CANTON CHRISTIAN COLLEGE

From the fall of 1916 to the summer of 1919, considerable data in regard to the Chinese water buffalo as a dairy animal was collected by the writer while on the agricultural staff of the Canton Christian College, Canton, China. Some of this data has been published in various journals and bulletins (4).

EXPLANATION OF MILK ANALYSES AND RECORDS

The following tables give the analysis of milk and production records of cows for which we have records for entire lactation periods.

Butter fat analyses were made twice a month with a Babcock centrifugal tester. The morning and evening milk was tested separately. The averages of the two tests was taken as the average test for the month. The milk for twenty-four hours was weighed twice a month. The average amount of milk for the two weighings was taken as the average daily production for the month.

The total solids (consisting of the fat, sugar, proteids, and ash) were found by evaporating a weighed sample of milk in a steam bath until the weight became constant. The ash was determined by heating in a crucible over a gas flame until the weight

became constant. The proteids were determined by the Kjeldahl method as described by Hawk in his Practical Physiological Chemistry, fourth edition, pages 410 and 483. The sugar was found by subtracting the sum of the ash, proteids and fat from the total solids. The percentages in each case were found by dividing the weight of the final product by the weight of the sample of milk analyzed.

Analysis and records of buffalo milk are all with cows in the college dairy.

The buffalo cows were fed a grain ration consisting of two pounds wheat bran and one pound rice chop. Each cow was fed about a pound of this mixed feed a day for each pound of milk given daily. The rice was fed cooked. The grain was fed separately to each cow twice a day. About 1½ ounces of salt was fed daily to the cows. The salt was mixed with the grain. About 40 pounds of water was mixed with the rice chop and bran at each feeding, making a very wet feed, the cows drinking it down rather than eating it. (This is the usual method of feeding grain to cattle in China.) The cows were fed all they could consume four times a day of a mixture of green cut grass, which amounted to from 40 to 80 pounds a day per cow.

Table 1 brings out a number of important facts about the milking qualities of the water buffalo in China. The average percentage of fat for the six cows for which records throughout a lactation period are available, was 11.25. The average amount of milk produced was 2293.3 pounds and the average amount of fat was 250.85 pounds, the highest being 326.31 pounds for cow 54. Another important fact brought out in these records, as shown in table 1, is the long lactation periods of the buffalo. As a rule, the cows in the college herd were milked for from eight to twelve months, depending on how soon after freshening they were bred. They are usually bred three months after freshening. When fresh, they give from 5 to 15 pounds a day, which diminishes very gradually throughout the long lactation period.

The production capacity of the cows in this experiment is striking when we consider the fact that the ten cows used were ordinary village cows of the beef-draft type, whose ancestors had never been used for dairy purposes.

TABLE 1

Analysis of milk; length of lactation period, amount of milk, amount of fat, and percentage of fat of the Chinese water buffalo milk

COW NUMBER	LENGTH OF LACTATION PERIOD	TOTAL AMOUNT OF MILK	TOTAL AMOUNT OF FAT	AVERAGE PERCENTAGE OF FAT
	days	*pounds*	*pounds*	
51	330	2863.4	278.22	9.81
53	277	1909.0	211.93	11.10
54	365	2644.5	326.31	12.33
55	253	1871.5	227.38	12.15
60	273	1849.0	203.68	11.02
63	325	2322.4	257.60	11.09

TABLE 2

Complete analyses of buffalo milk: The samples analysed were herd samples taken from twelve cows in the college dairy in November, 1917. The milking in the morning was begun at four, and in the afternoon at two o'clock

SAMPLE NUMBER	FAT	ASH	PROTEIN	SUGAR	TOTAL SOLIDS	WATER
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. Morning milk	11.00	0.94	6.04	4.00	21.98	77.02
2. Afternoon milk	12.80	0.90	6.10	3.57	23.37	76.65
3. Afternoon milk	13.00	0.71	5.71	3.32	22.74	76.98
4. Afternoon milk	13.63	0.74	5.94	3.57	23.92	77.08
5. Afternoon milk	12.10	0.90	6.14	3.83	22.87	77.04
6. Afternoon milk	14.00	0.92	6.42	3.87	25.21	74.79
7. Morning milk	11.50	0.95	5.80	3.70	21.95	77.05
8. Morning milk	12.00	0.94	6.00	3.60	22.54	77.45
9. Morning milk	12.34	1.04	6.28	3.71	23.10	76.63
10. Morning milk	12.20	0.77	5.90	4.23	23.02	76.98
Averages	12.46	0.89	6.03	3.74	23.29	76.89

As seen by table 2, the milk contains a great deal more proteids, sugar, somewhat more ash, and nearly twice as much solids as does European cow's milk.

ANALYSIS AND PRODUCTION OF THE DELHI (INDIAN) DAIRY BUFFALOS

In the region of Hongkong, there are a number of Delhi dairy water buffalo herds owned and managed by Indians. Dr. Adam Gibson, the Colonial Veterinarian, of Hongkong, who is also milk inspector for that territory, has informed the writer that the per cent of fat in the milk of this breed is similar to that of the Chinese water buffalo. According to British authorities in India, this breed in India commonly gives from 30 to 50 pounds of milk a day (3).

In January, 1919, the writer visited an Indian water buffalo dairy in Kowloon, which at that time had a total of twenty cows giving milk. The average production for the twenty cows was 15 pounds. Some of the cows had recently freshened, while others were in the seventh and eighth month of the lactation period. The feed at that time consisted of dry rice straw for roughness, and for concentrates they received a small amount of cooked rice chop and wheat bran. The cows were crowded into a dark, damp, poorly ventilated and dirty barn. With proper care, and the addition of succulent feed in the ration, the production of this herd could no doubt easily be doubled.

CHARACTERISTICS OF THE BUFFALO MILK OTHER THAN THAT SHOWN BY ANALYSES

Water buffalo milk is pure white in color. Butter made from the milk is also pure white. It is wholesome and very palatable when produced under sanitary conditions. Students and teachers (both European and Chinese) at the Canton Christain College prefer water buffalo to European cow's milk. On account of it having a high per cent of solids, it has a richer flavor, and European cow's milk seems thin and watery after drinking buffalo milk.

DISEASES OF THE WATER BUFFALO

Among water buffalos, as well as among the cattle of the Orient, probably the most common disease is a contagious disease known as rinderpest. The disease is somewhat like the chronic form of hog cholera, in that it is usually accompanied with fever and causes lesions in the inner lining of the intestines, but it is not as fatal as is cholera among hogs. Fortunately, a method of immunizing with anti-rinderpest serum has been worked out by veterinarians in the Bureau of Agriculture of the Philippines which is now being successfully applied in most parts of the Philippines, and used to some extent in India and Hongkong.

Tick fever, commonly known in the United States as Texas fever, after the region in which it is most common in the United States, is prevalent in South China. The disease is fatal to cattle imported from tick-free regions. Fortunately ticks do not travel far and breed only in grassy places. European cattle, in southern China, are usually kept in barns and in dry lots and suffer very little from the tick. The tick fever seems to have little or no effect on the water buffalos. They are apparently very highly resistant to the disease.

TUBERCULOSIS

According to Dr. Heanley of the Hongkong Bacteriological and Vaccine Laboratory, tuberculosis has never been found among the buffalo of south China, and in thirteen years' inspection of animals and carcasses in the Hongkong government slaughter house, where all animals slaughtered for food are inspected by government inspectors, only two cases in the humped cattle have come to notice. Both cases were bullocks. The disease is as common among European cattle as it is in America. Dr. Gibson, who has inspected the carcasses of buffalos slaughtered in Hongkong for the past thirteen years, at the rate of several hundred per week, confirms Dr. Heanley's report in stating that he has never seen tuberculosis lesions in the water buffalo.

Dr. M. Prettner, made a careful study of the resisting power of the water buffalo against experimental tuberculosis (5). Only when he injected tubercular material a number of times in the same buffalo, in amounts many times that required to induce tuberculosis in a cow or goat, could he produce the disease in the buffalo. In fact, even then, upon post-mortem, it was found that the organisms were found only in an inflamed area around the point of insertion of the tubercular material. The buffalos also had to be kept in an underfed and run-down condition in order to produce this slight infection. He reports that among five thousand buffalos slaughtered for meat, no case of tuberculosis was observed.

THE BUFFALO COMPARED WITH THE EUROPEAN COW AS A DAIRY ANIMAL

There are a number of commendable features in the use of the buffalo cow as a dairy animal. The amount of butter fat in milk in the better buffalo cows of China (see production tables) is not insignificant when we consider that there has been no breeding for production. Ordinary cows give more than two thousand pounds of milk and 150 to 300 or more pounds of fat a year. The "ram's horn" buffalo from India rank with the best breeds of modern dairy cattle, both in production of milk and total butter fat. The fact that the buffalo has no sweat or oil glands in the skin makes it an easy animal to keep clean. The scant hair on the body affords a poor hiding place for lice, and they can be easily detected and gotten rid of. The absence of tuberculosis among buffalos, and their resistance to the tick fever adds to their value as dairy cows. It is the writer's opinion that in regions where the water buffalo is thriving, the breeding of the native buffalo for production, and the importation of Indian dairy buffalos should be encouraged in every way possible, rather than encourage the importation and breeding of European cows, because of the danger from tuberculosis in the European cows.

OBJECTIONS TO THE BUFFALOS FOR DAIRY PURPOSES

In China, one of the chief reasons why so few buffalo cows are used for dairy purposes is no doubt because of the fact that they give but little milk, and that, while it contains about three and one-half times as much fat, and nearly twice as much total solids as does European cow's milk, it usually sells for the same price as the latter. However, as soon as the public knows the value of buffalo milk, it should command a much higher price than at present. Also the fact that cows which have not been especially bred for milk production, but simply selected from among ordinary buffalo herds, produce as much as 10 pounds of milk a day for several months, promises individuals giving a much larger amount of milk, with a few generations of selection and breeding for dairy purposes.

In warm weather, buffalo cows need a bath twice a day in order to keep at their best. It is the custom for them to be driven for about one-half hour each day to canals or ponds, which are not always clean for this wetting. To overcome this objection, tanks might be constructed in which clean water could be kept for their bath, or they may be wet twice a day by pouring clean water over them with buckets or with a hose where water pressure can be secured.

THE FUTURE OF THE BUFFALO IN DAIRYING

In India the buffalo is the chief source of milk where it is not only competing with good native breeds of the "humped" variety, of cattle but also with modern breeds of European dairy cattle.

There is a strong prejudice among Europeans in India against the use of buffalo milk because of the unsanitary methods under which it is frequently produced. In much of India, the buffalo is a scavenger, subsisting largely on not only the refuse of the kitchen, but also on horse dung, and even on human waste. Naturally the flavor of milk produced from such feed has an uninviting odor and flavor. With the education of the village farmers in better feeding methods, this condition will no doubt be improved.

It is the opinion of the writer that the water buffalo is bound to become an important dairy animal in the southern half of China as well as an important source of milk for the four hundred million people of that vast country. Unlike the Indians, the Chinese have not been users of milk in the past, but are rapidly taking to the use of this beverage through the example set by Europeans. With a few generations of intelligent selection and breeding among the beef-draft buffalo of China, there will be developed a dairy breed of high producing ability. The possibility of this is shown at the Canton Christian College by the fact that with no breeding or selection cows from an ordinary village herd, whose ancestors had never been milked, gave two thousand and more pounds of milk containing as much as two hundred sixty pounds of fat in less than one year.

The history of both the dairy and beef breeds of European cattle in most parts of the Philippine Islands has been little more than a keen disappointment and failure. The climate and diseases of most parts of the Islands are such that European cattle, as a rule, quickly succumb to diseases or degenerate from generation to generation. Two years ago, the Philippine legislature appropriated a large sum of money for the improvement of live stock in the Island. Very little of this money was used for importing more European cattle, but on the other hand, 200,000 pesos were set aside by the Bureau of Agriculture and the College of Agriculture for the importation of the Delhi dairy buffalos from northern India.

It is difficult to prophesy as to the future of the buffalo in the United States, if it is to have a future in this country. There is no doubt but that the buffalo would thrive in most parts of the south as far north as the southern part of Oklahoma. Most regions further north would probably be too cold. The swamps and marshes of Florida, Louisiana, and Mississippi should be especially adapted to buffalo production.

The fact that the buffalo is free from tuberculosis, as well as an excellent producer of milk and butter fat, may result in an attempt to introduce certain of the more distinct dairy breeds to the southern states. However, extreme care will need to be

used in preventing the introduction with the animals of diseases which have not yet found a footing in this country. Further study of the buffalo in its native land should be made in order to become better acquainted with its diseases and other problems connected with buffalo raising before the attempt to raise buffalos in America is made.

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THE RELATIVE VALUE OF THE METHYLENE BLUE REDUCTION TEST, THE BROMTHYMOL BLUE TEST, AND THE BROMCRESOL PURPLE TEST IN DETERMINING THE KEEPING QUALITY OF MILK[1]

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The various factors that determine the quality of milk have been presented in papers published in previous numbers of this Journal (1) (2). In the latter paper it was suggested that the consumer is interested in the richness, safety, cleanliness, and sweetness of the supply. The healthfulness and keeping quality are dependent on the bacterial content of the milk. The extent to which foreign matter has been introduced into the milk is also reflected in some degree by its bacterial content. The bacterial flora of every sample of milk is very complex, for many kinds of organisms are introduced from varied sources. Those which are unable to grow in milk can be of no importance in influencing its keeping quality. The detection of these types, however, may be of value in judging the quality of the milk, since they give some indication as to the extent to which it has been contaminated with foreign matter.

The forms that can grow in milk influence its keeping quality in varying degrees, the extent of which is determined by the temperature at which they grow most rapidly and by the nature of their by-products. If they are unable to grow at the temperatures at which milk is handled commercially, they can have no influence on its keeping quality. Again, if the chief by-products of an organism are carbon dioxide and hydrogen, as is apparently the case with certain of the bacteria of the B. lactis

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aerogenes group, the organism will be a minor factor in determining keeping quality as compared to one that grows equally well in milk, and whose chief by-product is lactic acid.

The by-products of different kinds of bacteria growing in milk may be of such a nature that they will neutralize each other, as for example alkali may be neutralized by the formation of acid. Again the various groups of bacteria react upon each other in different ways, some being retarded by the associated organisms, while others are favored, a relation generally referred to under the expression "associative action."

To obtain any idea of the effect of this complex of bacteria in influencing the keeping quality of milk is a task that can not be accomplished with any degree of completeness, no matter what method may be called into service. It seems to the writers that some have been unduly optimistic in regard to the methods they have suggested for the purpose of measuring the quality of milk, and in some instances they have not had in mind the various relations, briefly mentioned above, that may exist between different bacteria and between their by-products in milk.

The desirability of a method that will enable the analyst, within the shortest possible time and with the minimum of labor and expense, to place a number of samples of milk in the same order, with reference to keeping quality, as he would place them by a detailed examination of the milks after they have been kept for twenty-four hours at 50° or 60°F. is beyond question. In other words the method should enable the analyst to take into account all those points which the milk consumer uses in judging whether the milk delivered to him is to be graded as good or poor. Among these will be the development of acid, the occurrence of abnormal odors and tastes in milk that is not acid to taste or smell, and the ease with which the milks curdle on heating. No one method can accomplish this. The problem is then to select from the various methods available the one that approximates the ideal most closely. The method must be applicable to both raw and pasteurized milk. The information it will supply may differ in value, this being dependent on the class of milk to which it is applied. These matters have been

considered more in detail in a previous article (3). To the methods there discussed should be added two that have been recently suggested (4) (5). The authors of the first of these papers state that it was hoped to find a way by which to measure the ability of the bacteria and enzymes present to bring about changes in milk which might make it unsuitable for the purpose for which intended, and which method would also take into account the associative action of bacteria in milk, something other methods fail to do. The method suggested by these investigators involves the inoculation of plain broth containing bromthymol blue, adjusted to a reaction of pH 7.0, with 0.1 cc. of milk to be examined and its subsequent incubation at 37°C. The rate of change of reaction in the broth is determined by reading its hydrogen ion concentration at definite times. It is at once evident that when a mixture of bacteria such as is present in milk, is seeded into a medium so different chemically and physically from milk as is plain nutrient broth the relations existing between the groups will not continue to be the same as they were in the milk at the time of the transfer or as they would have been in the milk at any moment in its future. The changes which the bacteria produce in broth are therefore not likely to be the same, or even to correlate in intensity with those which the same mixture of bacteria would cause in milk. The data presented in the paper are not especially convincing as to the value of the method in determining the keeping quality of milk.

The method outlined in the second paper involves the addition of an indicator, bromcresol purple, to the milk to be examined and noting the change in color after a period of incubation. The indicator in question reveals the gradual change in reaction of the milk as acid is formed by the bacteria. It is evident that the method measures primarily the acid-forming bacteria. Since these are the prime factors in determining the keeping quality of milk, it is quite possible that the results obtained may correlate well with the keeping quality. The authors suggest the use of four stages or degrees of acidity: the first is indicated by the first observable change from the

grayish-blue noted in normal milk with this indicator to a lighter color in any part of the milk; the fourth stage is one in which the color of the mixture of milk and indicator becomes a yellow, free from every trace of bluish or greenish tinge. The curdling of the milk usually occurs at this last stage. The four stages therefore include perfectly sweet milk at one extreme and milk that is curdled at the other. The commercial life of milk is practically at an end when the increase in acidity amounts to a few hundredths of one per cent. The bacterial content of such milk is so high that its keeping quality, when stored at 18 to 20°C, is measured usually by a few hours. The method is not one by which milks, still commercially valuable, i.e., those in which no appreciable acidity has developed, can be differentiated into a number of groups.

It may be asked whether either of these methods is as delicate, any more easily worked, or less expensive than some of the methods that have been in use for a considerable time. The methylene blue reduction test has been shown by one of the writers (3) to be an excellent way of determining the bacterial content of milk. From the data presented, the conclusion was drawn that it is as delicate as the plate culture method, and possibly even more delicate. The reduction test has all the advantages of the methods suggested in the papers to which reference has been made. By its use the milks can be placed in many groups, the only limit being the differences in the milks, and the frequency with which they are examined during the period of incubation. If the milks are examined every fifteen minutes during the first four hours and at thirty minute intervals thereafter during a second period of four hours, they could be placed in twenty-four groups. Less frequent examination would result in the milks being thrown into a smaller number of groups.

The reduction test apparently is influenced by all the bacteria that are growing in the milk at the temperature employed, since so far as is known all bacteria reduce methylene blue. It may be that even the living cells that are not reproducing exert an influence in this test. Some data have been obtained that would indicate this possibility. It is probable that the reduction test

reflects the interrelations of the various groups of bacteria on each other far more perfectly than does the method proposed by the Michigan workers, since the test is made in the milk in question and not by transferring a part of it to a medium which has different chemical and physical properties. The method proposed by the New York workers measures but one group of bacteria, the acid-formers. A method which measures the number of bacteria rather than their by-products should theoretically possess greater value in the examination of milk than a method which measures the by-products. From theoretical grounds the reduction test seems to approximate the ideal method more closely than does any other method.

It is not intended to convey the idea that the reduction test is influenced to the same extent by all bacteria. It will be shown in a later paper that all the bacteria in the milk are concerned in bringing about the reduction of the methylene blue. Furthermore, the application of the test itself is not possessed of the difficulties and uncertainties of the other two methods, namely, that of detecting delicate color changes either with the aid of a comparator or with the eye alone. Memory for delicate distinctions in shades of a color may easily change from day to day while it is doubtful that there would be any hesitation in deciding between color and absence of color as is the case in the end point for the reduction of methylene blue.

For the purpose of determining the relative value of the reduction test as compared with the use of bromcresol purple in milk, and bromthymol blue in broth inoculated with milk, several comparative tests were made. Pure cultures of typical milk organisms were used, and by means of definite dilutions an approximate idea was had of the relative number of organisms present in the samples tested. The methods used and the results obtained for the several trials were so similar that only a few representative comparisons will be given.

EXPERIMENTAL

One hundred and forty cubic centimeter portions of fresh whole milk which had been heated to 100°C. for a short time were inoculated separately with 1 cc. of an eighteen hour old milk culture of the organism to be tested. Several strains of B. coli and of B. lactis aerogenes, and a few cultures isolated from milk, two of which are numbered 20.2 and 44.10 were used. From each 140 cc. of inoculated milk, nine 15 cc. portions were measured out. The first 15 cc. portion of each culture was inoculated with 1.5 cc. of a freshly curdled culture of Bact. lactis acidi, and of this dilution 10 cc. was transferred to another 15 cc., which method of dilution was continued until all nine of the 15 cc. portions of inoculated milk of the different series had received diminishing amounts of the lactic culture. A set of the different dilutions of the lactic culture alone was made in uninoculated heated milk. Each series therefore included eight samples of milk, decreasing in bacterial content from the first to the last. In the set in which Bact. lactis acidi alone was present the dilution was as follows:

Sample 1.	1 part of culture to	25 parts of milk
Sample 2.	1 part of culture to	62 parts of milk
Sample 3.	1 part of culture to	155 parts of milk
Sample 4.	1 part of culture to	390 parts of milk
Sample 5.	1 part of culture to	975 parts of milk
Sample 6.	1 part of culture to	2,437 parts of milk
Sample 7.	1 part of culture to	5,092 parts of milk
Sample 8.	1 part of culture to	12,730 parts of milk

In the sets in which Bact. lactis acidi was used with another organism, the inoculum of the latter was the same in each sample, while the lactic organism varied in number as described above. The result was a series of samples of milk of a gradually decreasing bacterial content. An accurate method of measuring the number of cells in the various samples in any set should place the samples in the order of their bacterial content.

Methylene blue reduction tests, bromthymol blue broth tests, and bromcresol purple milk tests were run on each dilution except the first. For the reduction test, 1 cc. of Merck's methylene

blue (1:20,000) was added to 10 cc. of the inoculated milk and reduction was carried out in a water bath at 37°C.

For the bromthymol blue test sterilized beef-peptone broth (pH of 7.0) to which had been added the indicator in the proportion of 4 drops of a 0.02 per cent solution to each 10 cc. of medium, was pipetted in 10 cc. portions into sterilized test tubes. One-tenth cubic centimeter of the milk to be tested was added direct to the broth and the tubes were incubated in a 37°C. water bath. Color controls were made by adding an equal amount of heated uninoculated milk to 10 cc. of the bromthymol blue broth, and changes in reaction were determined at the end of one and two hours incubation by comparing the inoculated tubes with the uninoculated for any variation in color. What is reported in table 1 as a slight change was not more than 0.1 in the pH scale of hydrogen ion values.

The bromcresol purple test was made by adding 5 cc. of the milk dilutions of the cultures to sterilized tubes containing 0.05 cc. of the indicator (1 part in 1000 parts of water). These were also incubated at 37°C. and readings were made at the end of one, two and twenty-four hour periods for any variation in color from that of the control.

In table 1 the changes in bromthymol blue and bromcresol purple are recorded only after the two hour incubation, because most of the cultures in practically all of the dilutions showed no change at the end of one hour. These two indicators were not influenced by the number of bacteria except where this was very marked, as in the first dilutions, whereas the reduction time of methylene blue in all cases tended to vary directly according to the number of organisms present. When growth took place throughout the whole of a series of dilutions in broth, the change in reaction, as shown by the color of the indicator, was the same for all except usually the first dilution. No such gradation of milks, based on the number of bacteria present, as was obtained with methylene blue, ever resulted with the two indicators bromthymol blue and bromcresol purple. The bromcresol purple has shown in two hours time even less gradation than the bromthymol blue, and when incubation was continued for twenty-

TABLE 1

Comparison of the methylene blue reduction test, the bromthymol blue test, and the bromcresol purple test on milks inoculated with varying amounts of pure cultures

	SAMPLE							
	1	2	3	4	5	6	7	8
Bact. lactis acidi								
Methylene blue reduction time in minutes	6	30	57	117	162	222	283	358
Bromthymol blue after 2 hours at 37°C.	++	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	++	−	−	−	−	−	−	−
Bact. lactis acidi and culture 20.2								
Methylene blue reduction time in minutes	7	29	52	61	71	81	90	105
Bromthymol blue after 2 hours at 37°C.	+++	+++	+++	+++	+++	+++	+++	+++
Bromcresol purple after 2 hours at 37°C.	+	−	−	−	−	−	−	−
Bact. lactis acidi and culture 44.10								
Methylene blue reduction time in minutes	5	24	52	71	85	102	103	118
Bromthymol blue after 2 hours at 37°C.	+++	++	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	++	+	−	−	−	−	−	−
Bact. lactis acidi and B. coli								
Methylene blue reduction time in minutes	7	21	52	71	93	115	131	190
Bromthymol blue after 2 hours at 37°C.	+	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	+	−	−	−	−	−	−	−
Bact. lactis acidi and B. aerogenes A_2								
Methylene blue reduction time in minutes	7	29	63	89	96	110	131	147
Bromthymol blue after 2 hours at 37°C.	++	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	+	−	−	−	−	−	−	−

TABLE 1—*Continued*

	SAMPLE							
	1	2	3	4	5	6	7	8
Bact. lactis acidi and B. aerogenes B_2								
Methylene blue reduction time in minutes	6	29	58	82	100	118	142	156
Bromthymol blue after 2 hours at 37°C.	+++	+++	+++	+++	+++	+++	+++	+++
Bromcresol purple after 2 hours at 37°C.	–	–	–	–	–	–	–	–

– No change; + very slight; ++ slight change; +++ decided change.

four hours the maximum color change of the indicator was reached in all the different dilutions.

The conclusion to which one is forced is that neither of the methods is as delicate nor as accurate a measure of the bacterial content of milk as is the reduction test. They did not enable the analyst to place the varying dilutions in their proper order in respect to the number of bacteria while the reduction test failed in this in no instance. The few data presented are typical of all collected in making over two hundred such trials.

In order to compare the three tests on milks of unknown bacterial content, market milks which were anywhere from four to twenty-four hours old were used. In making the bromthymol blue test, the inoculum for the broth was 1 cc. of a 1 to 10 dilution of the milk in water instead of 0.1 cc. of milk as previously used. For the bromcresol purple test 5 cc. of milk and 0.05 cc. of indicator were used. The results are reported in tables 2 and 3.

With these milks the results were similar to those obtained with pure cultures in milk; neither of the indicators was so sensitive to the biological differences in the milks as was the methylene blue. The bromthymol blue test when applied to milks of widely different keeping quality, showed in a general way rough agreement with the methylene blue, but it would not divide the milks into as many classes as did the latter. The tubes of broth inoculated with milks which were at the curdling point did not show a change in reaction in an hour while the

reduction time for such milks is very short, a minute or less. In the case of milks that had a reduction time of two to thirty minutes, which means that such milks have passed the point where they are marketable, from two to five hours were required

TABLE 2

Methylene blue reduction time of milks compared with time for first change in bromthymol blue broth

SAMPLE OF MILK	METHYLENE BLUE REDUCTION TIME	BROMTHYMOL BLUE COLOR CHANGE
	minutes	*minutes*
1	1	75
2	2	285
3	3	135
4	3	300
5	4	300
6	8	180
7	11	154
8	21	270
9	27	300
10	30	300
11	36	285
12	58	300
13	200	300
14	240	420
15	280	300
16	295	300
17	360	480
18	420	No change in 480
19	450	540
20	480	600, change doubtful
21	480	600, change doubtful
22	510	No change in 600
23	540	600
24	540	540
25	570	No change in 650
26	Slight reduction in 570	600
27	Slight reduction in 570	No change in 600
28	Slight reduction in 570	600, change doubtful
29	No reduction in 570	540
30	No reduction in 570	540

before a change was noted in the color of the bromthymol blue broth. Milks with a reduction time of eight to ten hours required only eight to ten hours for a change in color of bromthymol blue to become evident.

The variations in the color itself of different milks cause variations in the color of the bromthymol blue broth to such an extent that considerable error may occur unless a control tube is made from each milk tested with which the sample that has been incubated can be compared.

TABLE 3

Methylene blue reduction time of milks compared with their color reaction with bromcresol purple

SAMPLE OF MILK	METHYLENE BLUE REDUCTION TIME	BROMCRESOL PURPLE COLOR CHANGE
	minutes	
1	1	Yellow
2	1	Yellow
3	9	Lighter than control
4	23	Lighter than control
5	46	Same as control
6	84	Greenish tinge
7	134	Greenish tinge
8	136	Same as control
9	189	Darker than control
10	189	Lighter than control
11	240	Darker than control
12	260	Lighter than control
13	280	Darker than control
14	300	Greenish
15	314	Lighter than control
16	314	Greenish tinge
17	314	Lighter than control
18	344	Same as control
19	350	Same as control
20	No change in 8 hours	Slightly lighter than control
21	No change in 8 hours	Slightly lighter than control
22	No change in 8 hours	Slightly lighter than control

The failure of the bromthymol blue broth to differentiate between milks to the degree that the methylene blue does might be attributed to several things. Diluting milks of a varying bacterial content might tend to overcome the variations. Instances are not uncommon in plate cultures where a dilution falls far short of having the theoretical number of organisms when compared with the preceding dilution. As suggested be-

fore, rearrangement of the relation among the different groups of organisms present in a milk both by its dilution and by its inoculation into a different medium, and subsequent adjustment to a new environment would also tend to overcome the original variations in the milks.

The bromcresol purple test when used with milks ranging from a few to twenty-four hours old brought out marked differences, but conclusions as to their keeping quality based on these differences could not be relied upon because of the numerous factors, such as color of the milk, fat content, and total solids, which may influence the color of this indicator. Examples were found of milks which, according to their methylene blue reduction time, would be at opposite ends when classified in respect to their keeping quality, gave with bromcresol purple the same color. The light color with the two milks reducing in nine and twenty-three minutes, table 3, was no doubt due to growth; but the same color in a milk with a reduction time of five hours was of course due to some factor other than growth. It is also quite probable that acid in amounts sufficient to be detected by bromcresol purple was formed in a milk with a reduction time of forty-six minutes and could have been detected by this indicator provided a control tube of the same milk in which no growth had taken place had been available. Thus it is evident that large numbers of bacteria may grow in some milks without changing the color of the indicator to the degree that an acid reaction can be detected, and it is even possible that in some milks the hydrogen ion concentration might not be changed though considerable growth had taken place. Especially would this be the case in a milk high in total solids and basic salts where any acid that was formed would be taken up by these constituents. Furthermore, a neutral reaction might be maintained in a milk for a considerable length of time because of the balance produced by the growth of acid- and alkali-producing organisms. Such a milk would have a high bacterial content before the acid formers would affect the color of the indicator. If a milk were low in total solids, it would give a darker blue with bromcresol purple than a normal milk and in order to bring about the same

color in the acid direction in the two milks, it would require in the case of the abnormal milk either the growth of a greater number of acid-producing organisms or a longer period of growth than would be required in the milk having a normal color at the start.

CONCLUSION

The results herein reported show that the methylene blue reduction test is a more accurate measure of the number of bacteria in milk than is either the bromthymol blue or the bromcresol purple test. These two tests depend on the measurement of acid, one of the by-products of the growth of bacteria, whereas the reduction of methylene blue depends on the number of living cells.

The reduction test involves no more labor or time than do the other tests and is more easily carried out by untrained workers. And these in using either of the indicator tests would undoubtedly arrive at different conclusions in judging the quality of a series of milks because of the possible errors and difficulties in interpreting the tests. Interpretation of the methylene blue test might in a few instances present difficulties, but these have been met with so seldom that they might be considered negligible. It is to be expected that the methylene blue test in its application will reveal some discrepancies, but these are not, in the experience of the writers, as obvious as those in the bromthymol blue and the bromcresol purple tests.

In a subsequent paper it will be shown that different kinds of bacteria do not have the same reducing power; and that the lactic acid bacteria which are of the greatest importance in the spoiling of milk are also most active in the reduction of methylene blue, while other common milk bacteria such as those of the B. coli-aerogenes group which are of secondary importance in the spoiling of milk, are likewise less active in the reduction of methylene blue. It may well be, therefore, that the reduction test will prove to be an excellent measure of the keeping quality of milk.

SUMMARY

In a comparison of the three tests, methylene blue reduction, bromthymol blue, and bromcresol purple for the determination of the keeping quality of milk, it is shown that the methylene blue reduction test is the preferable because of its greater sensitiveness to biological differences in milks; because it measures the number of bacteria rather than the by-products of their growth; and because of its simplicity and practicable advantages.

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THE DIGESTIBILITY OF SORGHUM MILL REFUSE[1]

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The sorghum mill was at one time a common institution in many sections but in prewar days the number in operation had become greatly reduced. The sugar shortage during the last few years has again led to the operation of a large number of them. The amount of sorghum grown for syrup is not great in this section but yet questions regarding the feeding value of the sorghum mill refuse are frequent.

No information is available on this point so the present work was undertaken to determine the value of this product as a feed for livestock.

EXPERIMENTAL METHODS

The sorghum refuse was obtained from a local mill and consisted of the stalks remaining after the extraction of the juice. It was generally about two days old when used and was in quite fair condition. It was fed uncut.

The length of the feeding period was 12 days and during the last five days the digestion trial was conducted according to the methods generally recognized. Salt was given the animals at free will and they were watered twice daily.

The work was started on October 5, 1919, and where necessary the information given concerning the animals used is calculated to that date. Cow 91 was a purebred Ayrshire and cow 262 was a Guernsey-Ayrshire cross. Both animals had been used for a considerable time in other feeding and digestion trials.

The weights of the feed consumed and the faeces produced were determined daily. The faeces samples were dried and composited and these with the samples of air dried feed were the materials on which the proximate constituents were determined

[1] The coöperation of W. G. Gaessler in undertaking the analytical work for this project deserves acknowledgment here.

TABLE 1

Animals used

	cow 91	cow 262
Age	12 y., 1 m., 3 d.	5 y., 2 m., 18 d.
Days bred	0	158
Weight, pounds	1000	1080
Previous lactations	6	1

TABLE 2

Feed consumed and faeces produced

	cow 91	cow 252	AVERAGE
	pounds	*pounds*	*pounds*
Feed	78.0	92.2	85.1
Faeces	97.5	105.7	101.6

TABLE 3

Analyses of feed and faeces

	SORGHUM MILK REFUSE	FAECES		
		Cow 91	Cow 262	Average
	per cent	*per cent*	*per cent*	*per cent*
Moisture	73.12	85.27	84.74	85.00
Dry matter	26.88	14.73	15.26	15.00
Crude protein	0.62	1.09	1.21	1.15
Nitrogen-free-extract	12.98	7.01	7.19	7.11
Crude fiber	11.48	4.72	5.42	5.08
Crude fat	0.46	0.22	0.21	0.21
Ash	1.34	1.69	1.23	1.45

TABLE 4

Nutrients consumed, defecated and digested

	CONSUMED			DEFECATED			DIGESTED		
	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Dry matter	20.97	24.78	22.88	14.36	16.13	15.25	6.61	8.65	7.63
Crude protein	0.48	0.57	0.53	1.06	1.28	1.17	−0.58	−0.71	−0.65
Nitrogen-free-extract	10.13	11.97	11.05	6.84	7.60	7.22	3.29	4.37	3.83
Crude fiber	8.95	10.58	9.77	4.60	5.73	5.17	4.35	4.85	4.60
Crude fat	0.36	0.42	0.39	0.21	0.22	0.22	0.15	0.20	0.18

TABLE 5

Digestion coefficients and digestible nutrients

	DIGESTION COEFFICIENTS			DIGESTIBLE NUTRIENTS
	Cow 91	Cow 262	Average	
	per cent	*per cent*	*per cent*	*per cent*
Dry matter	31.5	34.9	33.4	9.0
Crude protein	0.0	0.0	0.0	0.0
Nitrogen-free-extract	32.5	36.5	34.7	4.5
Crude fiber	48.6	45.8	47.1	5.4
Crude fat	41.7	47.6	44.9	0.2

by the recognized methods. All analyses were then computed back to the moist basis and only the analyses of the original undried materials are presented here.

DISCUSSION OF RESULTS

The average coefficients of digestibility for the sorghum mill refuse were low, that of the dry matter being 33.4 per cent. The crude protein was apparently undigested due to the fact that the amount of protein present was not sufficient to maintain the nitrogen balance in the animals and the presence of metabolic products caused the elimination of more crude protein in the faeces than there was in the feed.

The amounts of digestible nutrients present are small, the total content being only 10.4 per cent. When the net energy value of the sorghum mill refuse is calculated by the method of Armsby[2] it is found to be only 6.5 therms or a lower net energy value than has been reported for any other feed.

SUMMARY

The digestibilities of the nutrients in sorghum mill refuse are low and the net energy value is exceptionally low so there appears to be little opportunity for the extensive use of this waste produce as a feed.

[2] H. P. Armsby and J. A. Fries. Net energy values for ruminants. 1916, Bul. Pa. Agr. Exp. Sta. 142.

THE DIGESTIBILITY OF CORN CANNERY REFUSE[1]

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The canning of sweet corn has now reached enormous proportions and huge quantities of refuse have to be disposed of in connection with these activities. A considerable quantity of this material is fed but a great proportion of it has in the past been allowed to go to waste. In recent years, however, the interest in this as a feed for farm livestock has been increasing and the work outlined here was undertaken with the object of obtaining some information regarding the value of the refuse from sweet corn canning factories. No work has as yet been reported on this problem and the material herein presented is merely indicative of the need for seriously considering the utilization of this waste product.

EXPERIMENTAL METHODS

The corn cannery refuse used was obtained from a local factory and was generally two days old when fed. It was partially fermented and not in first class condition for feeding purposes as there was no means of handling it at the factory except by dumping. It consisted mainly of the sweet corn husks with only small amounts of spoiled ears and cobs as these generally went into separate piles. The material, when fed, had a bad odor but was consumed in fair quantities by the animals used in the experiment.

The feeding period was of twelve days duration and during the last 5 days the digestion trial was conducted according to the recognized methods. Salt was given at free will and the animals were watered twice daily.

The work was started on September 15, 1919, and where necessary the data given concerning the cows is calculated to that date. Cow 91 was a purebred Ayrshire while cow 262 was a

[1] The coöperation of W. G. Gaessler in undertaking the analytical work for this project deserves acknowledgment here.

Guernsey-Ayrshire cross and both had been used for a considerable time in feeding trials previous to being used for this work.

The weights of feed consumed and faeces produced were obtained daily and the faeces samples were dried and composited.

TABLE 1

Animals used

	cow 91	cow 262
Age	12 y., 0 m., 13 d.	5 y., 1 m., 28 d.
Days bred	0	138
Weight, pounds	1005	1090
Previous lactations	6	1

TABLE 2

Feed consumed and faeces produced

	cow 91	cow 262	Average
	pounds	*pounds*	*pounds*
Feed	219.75	249.50	234.63
Faeces	182.75	158.00	170.38

TABLE 3

Analyses of feed and faeces

	CORN CANNERY REFUSE	FAECES		
		Cow 91	Cow 262	Average
	per cent	*per cent*	*per cent*	*per cent*
Moisture	83.28	86.57	81.73	84.33
Dry matter	16.72	13.43	18.27	15.67
Crude protein	1.32	1.53	1.85	1.68
Nitrogen-free-extract	9.17	6.59	8.74	7.59
Crude fiber	5.02	4.38	6.20	5.22
Crude fat	0.34	0.16	0.40	0.27
Ash	0.87	0.77	1.08	0.91

The proximate constituents of these samples and the air-dry sample of feed were determined by the recognized methods. All analyses were calculated back to the moist basis and are reported here on the basis of the original materials.

DISCUSSION OF RESULTS

The average digestion coefficients for the corn cannery refuse were quite low, that for the dry matter being 31.9 per cent. Of the individual constituents, those occurring in the smallest amounts, namely fat and protein, showed the greatest variations. The average coefficient of digestibility for the fat was 42.5 per cent and that for the crude protein was only 7.7 per cent. In

TABLE 4

Nutrients consumed, defecated and digested

	CONSUMED			DEFECATED			DIGESTED		
	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Dry matter	36.74	41.72	39.23	24.54	28.87	26.71	12.20	12.85	12.53
Crude protein	2.90	3.29	3.10	2.80	2.92	2.86	0.10	0.37	0.24
Nitrogen-free-extract	20.15	22.88	21.52	12.04	13.81	12.93	8.11	9.07	8.59
Crude fiber	11.03	12.53	11.78	8.00	9.80	8.90	3.03	2.73	2.88
Crude fat	0.75	0.85	0.80	0.29	0.63	0.46	0.46	0.22	0.34

TABLE 5

Digestion coefficients

	cow 91	cow 262	Average
	per cent	*per cent*	*per cent*
Dry matter	33.2	30.8	31.9
Crude protein	3.5	11.2	7.7
Nitrogen-free-extract	40.2	39.6	39.9
Crude fiber	27.5	21.8	24.4
Crude fat	61.3	25.9	42.5

addition the apparent abilities of the animals so far as digestion was concerned varied greatly in the case of the fat and the protein, cow 91 having a digestion coefficient of 61.3 per cent for fat and 3.5 per cent for crude protein while the corresponding figures for cow 262 were 25.9 per cent and 11.2 per cent. The animals showed quite uniform ability so far as the digestion of the two chief constituents of the dry matter, nitrogen-free extract and crude fiber, were concerned.

A better appreciation of these results can perhaps be obtained if a comparison be made between the corn cannery refuse and corn silage. The comparison is made with corn silage rather than with corn stover silage as no digestion trials have been reported with stover silage.

When the comparison between the corn cannery refuse and corn silage is made it is found that the refuse contains about 10

TABLE 6

*A comparison of corn silage and corn cannery refuse**

	COMPOSITION		DIGESTION COEFFICIENTS		DIGESTIBLE NUTRIENTS		DIGESTIBLE NUTRIENTS IN 1 TON		
	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Cannery refuse and 5 bushels corn
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*			
Moisture	73.7	83.3							
Dry matter	26.3	16.7	66	32	17.4	5.3	348	106	
Crude protein	2.1	1.3	51	8	1.1	0.1	22	2	23
Nitrogen-free-extract	15.4	9.2	71	40	10.9	3.7	218	74	264
Crude fiber	6.3	5.0	65	24	4.1	1.2	82	24	27
Crude fat	0.8	0.3	82	43	0.7	0.1	14	2	15
Ash	1.7	0.9							
Carbohydrate					15.0	4.9	300	98	291
Carbohydrate equivalent					16.6	5.1	332	102	325
Total					17.7	5.2	354	104	348
Nutritive ratio							1:15	1:51	1:14

* The data on corn silage are from Feeds and Feeding, W. A. Henry & F. B. Morrison, 17th edition, 1917.

per cent more moisture than does the silage and so is correspondingly low in the valuable nutrients, this being especially true in the case of the fat. The coefficients of digestibility for the corn cannery refuse are much lower than those for the silage and the most marked decrease is found in the case of the apparent digestibility of the crude protein. This was probably due to the fact

that the relatively small amount of protein in the feed allowed the results to be masked by the metabolic products in the faeces.

The content of digestible nutrients differs widely for the two feeds, being 17.7 per cent of total digestible nutrients in the case of the corn silage and 5.1 per cent in the case of the corn cannery refuse. The relative difference is most marked in the case of the crude protein and fat.

Another method of comparing the two feeds is to study the amount of digestible nutrients in 1 ton of each. A ton of corn silage contains 22 pounds of digestible crude protein and 332 pounds of digestible carbohydrate equivalent or 354 pounds of total digestible nutrients, while 1 ton of the corn cannery refuse studied contained only 2 pounds of digestible crude protein and 102 pounds of digestible carbohydrate equivalent or 104 pounds of total digestible nutrients. In other words, 1 ton of corn silage contains about as much digestible nutrients as $3\frac{1}{2}$ tons of the cannery refuse. The nutritive ratio of corn silage is 1:15 while that of the refuse is 1:51.

The relative feeding values of the two feeds can not be properly appreciated from these figures but if 5 bushels of corn, the average amount in 1 ton of silage, be added to 1 ton of the refuse it is found that this combination will contain 23 pounds of digestible crude protein and 325 pounds of digestible carbohydrate equivalent or 348 pounds of total digestible nutrients. This means that 1 ton of corn cannery refuse and 5 bushels of corn will have a feeding value approximately equal to that of 1 ton of good corn silage.

SUMMARY

Large amounts of corn cannery refuse are allowed to waste but if proper methods of storage and handling were instituted a considerable amount of valuable feed could be saved as 1 ton of corn cannery refuse and 5 bushels of corn are apparently of about the same feeding value as 1 ton of good corn silage.

A BACTERIOLOGICAL AND BIOCHEMICAL STUDY OF EXPERIMENTAL BUTTERS[1]

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Originally, this investigation was intended as a study of the effects of cream ripening and of pasteurization upon the keeping qualities of butter. Owing to the fact, however, that considerable time has elapsed since the work was done and in this time some of the methods and ideas of manufacture have undergone some changes, it is thought best to present the results from the point of view expressed in the title.[2] The reasons for this decision will become clearer as the work is presented. It is hoped that the data to be presented will be of value to bacteriologists and chemists studying this problem, if not to practical butter makers.

While the purpose of this investigation was to study the effect of ripening and pasteurization on the keeping quality of the butter, the effects of other treatments of the butter were also to be observed. These included, (a) washing of the butter with lactic acid, (b) the addition of casein, (c) the addition of fishy butter, and (d) the addition of boric acid.

A single vat of cream was divided into two equal portions, one of which was pasteurized and the other unpasteurized. Each of these portions was further subdivided into two parts, one part being churned without further treatment and the other ripened by the addition of starter. Each of the four divisions of cream was then churned separately. The butter from each churning was then divided into five portions which were subjected to the four further treatments mentioned above, with one of

[1] Published by permission of the Director of the Michigan Experiment Station, East Lansing, Mich.

[2] The work presented here was done during 1909 and 1910 by Chas. W. Brown and Lulu M. Smith but was not published. Mr. Ruehle is not responsible for the plan of the work nor for the gathering of the data, but is largely responsible for the presentation of the results in written form.

the portions acting as an untreated control. The twenty different butters (about 60 pounds each) were packed in 30 pound tubs and shipped without delay to a cold storage plant where they remained for the duration of the experiment at about 0°F. At intervals of 9, 48, 164, 275, and 426 days the butters were scored and sampled for bacteriological and chemical determinations.

The following scheme indicates the divisions of the cream and the treatments of the butters:

			Sample	Treatments
Cream 28.18 per cent fat 53° acidity	Raw	Part 1, churned immediately, 53° acid	1 and 11	Control
			2 and 12	Acidified H_2O in 2d washing
			3 and 13	Casein added
			4 and 14	Fishy butter added
			5 and 15	Boric acid added
		Part 3, to 60°	21 and 31	Control
			22 and 32	Acidified H_2O in 2d washing
			23 and 33	Casein added
			24 and 34	Fishy butter added
			25 and 35	Boric acid added
	Pasteurized at 160°C.	Part 2, churned immediately	6 and 16	Control
			7 and 17	Acidified H_2O in 2d washing
			8 and 18	Casein added
			9 and 19	Fishy butter added
			10 and 20	Boric acid added
		Part 4, to 64°	26 and 36	Control
			27 and 37	Acidified H_2O in 2d washing
			28 and 38	Casein added
			29 and 39	Fishy butter added
			30 and 40	Boric acid added

A more detailed description of the methods of manufacture of the butters and the methods of bacteriological and chemical investigations follows.

SECURING THE CREAM

The cream was secured on a prearranged date (October 8, 1909) from three near-by creameries; from the first was received 1365 pounds of 28.91 per cent butterfat, from the second 1471 pounds of 26.93 per cent butterfat and from the third 1118 pounds of 28.93 per cent butterfat—a total of 3954 pounds of 28.18 per cent butterfat. The cream from the first creamery, all gathered cream, arrived in good condition; that from the second, all gathered cream, was sour when it arrived; that from the third, about half gathered and half whole milk cream, arrived in good condition. The three lots were placed in a cool room until the following morning then they were turned into a large vat and stirred to make a homogeneous mixture.

TREATMENT OF CREAM

The mixed cream in the vat contained 28.18 per cent butterfat and had an acidity of 53°. A slight metallic flavor, possibly derived from some of the cans used in shipping, was present. Part I was churned at once. Part III received an addition of 5 per cent starter (69° acid) and was held at 60°F. for about five hours then cooled to 50°F. and held over night. The ripened cream had an acidity of 60°. Part II was chilled immediately after it had been pasteurized at 160° in a Farrington pasteurizer and was churned about an hour later. No starter was added. Part IV received an addition of 5 per cent starter and was held for five hours at 60°F then cooled to 50°F. and held over night at that temperature. At time of churning the cream had an acidity of 64°.

CHURNING AND STORAGE

The churning was done in the College Dairy under the supervision of L. B. Liverence, in charge of dairy manufacture. Butter from each churning was treated as follows:

Butter washed with lactic acid. After the first washing, the butter (60 pounds) was placed in a small churn and 15 pounds of a 1 per cent commercial lactic acid solution was added. The

churn was given five revolutions and then the acidified water was allowed to drain away. Dry salt was added and the butter was worked sufficiently to incorporate it, after which the butter was packed into two thirty pound tubs.

Butter with added casein. To 60 pounds of salted butter in a small churn was added 90 grams powdered casein, prepared from sweet separated milk by precipitating with lactic acid and washing successively with water alcohol, absolute alcohol, and ether. The butter was worked to incorporate the casein—the water being allowed to drain away—and was packed into two 30 pound tubs.

Butter with boric acid added. One hundred and twenty grams of powdered boric acid was worked into 60 pounds of salted butter; the excess water was allowed to drain away and the butter was packed into two 30 pound tubs.

Butter with fishy butter added. One pound of fishy butter which was obtained from a tub of butter scored as having a pronounced fishy flavor by the Michigan Dairy and Food Department at a judging contest in Grand Rapids, was worked into 60 pounds of salted butter. The butter was placed in two 30 pound tubs. The fishy butter used in this work was analyzed bacteriologically and was found to contain 2,300,000 bacteria per gram. The flora isolated consisted of non-liquefying yeast, M. lactis rosaceus and a number of unidentified species.

Unmodified butter. The remainder of the churning was also tubbed; two 30 pound tubs being used as controls.

Storage of butter. The tubs were numbered and expressed immediately to the Booth Cold Storage at Detroit where a temperature was maintained at 0°F. Twenty-four hours before each scoring the tubs were placed in a comparatively warm anteroom to the cold storage, being returned to the storage room as soon as scored.

SCORING AND SAMPLING

When the butter was 9, 48, 164, 276 and 426 days old it was judged independently by F. O. Foster, G. A. Gilbert and L. B. Liverence. The average score with the descriptive terms used

are recorded in table 1, and will be discussed on page 380. At the time of each scoring two sets of samples were taken from one of each pair of tubs. For the first set about 50 grams were taken with a sterile trier, placed in sterile morphine bottles and used the following day for bacteriological analyses and for the determination of acidity, moisture and salt. For the second set of samples about 1 pound was taken with a knife, placed in mason jars—no surface butter was included—and was used the second day following for chemical analyses. All samples were kept cold from the time of collecting to the time of analyzing.

PLATING AND ANALYTICAL METHODS

The bottle containing the sample for bacteriological analysis was placed in a water bath at 35°C. until the butter softened. The cork was removed and the butter stirred with a sterile glass rod, then 11.5 cc. (10 grams) of butter were introduced by means of a sterile pipette into 90 cc. of warm (35° to 40°C.) sterile physiological salt solution; from this the higher dilutions were made. Nine plates were made from each sample; one set of three (three different dilutions) was poured with litmus lactose agar (standard meat infusion agar with acidity of 10°F.S. and containing 1 per cent lactose and 0.025 per cent azolitmin) and two sets were poured with lactose salt agar (same as above without azolitmin and containing 10 per cent sodium chlorid). The litmus lactose agar plates were incubated at 20° to 22°C. for five to seven days then isolations and counts were made. One set of lactose salt agar was incubated at 20° to 22°C. for ten to fourteen days and the other set at 5° to 10°C. for about twenty days. At this time isolations and counts were made.

The methods for the determination of acidity, moisture, salt, total nitrogen, nitrogen not precipitated by copper sulphate, and acidity of butter leachings are the same as those employed in a previous study (Technical Bul. 2, Mich. Agr. Sta., pp. 13–15) with the exception that the acidity of butter is recorded as cubic centimeters of N/10 acid per 100 grams butter.

Lactose was determined according to the method described by Benedict (1) (1907). One hundred cubic centimeters of the

filtrate from the copper sulphate precipitation is placed in a 100 to 110 cc. sugar flask, a few drops of phenolphthalein is added and N/1 sodium hydroxid is added drop by drop until the copper is precipitated (the phenolphthalein indicates when enough alkali is added). The volume is made up to 110 cc. and filtered. Ten cubic centimeters of Benedict's reducing solution are placed in a small Erlenmeyer flask and heated to boiling. The lactose solution is run in from a burette, the number of cubic centimeters required to reduce the copper solution is read and the amount of lactose is computed and recorded as percentage of butter.

RESULTS

The results of the scorings on flavor and keeping quality and of the bacteriological studies so far as the seedings made on lactose agar incubated at 19°C. are concerned, are presented in table 1. The results of the other platings will be discussed but not presented in full, owing to lack of space.

Table 1 needs a word of explanation. The scorings are presented under headings as "sample" and "duplicate sample." These are two tubs of butter in each case, as numbers 1 and 11, 2 and 12, etc. The bacterial and chemical determinations, however, were only made on one of the tubs and represent the butters listed under the heading "sample." The heading "flora isolated" should not be misinterpreted as meaning that the organisms listed were the only ones isolated. They represent the only ones which survived long enough to be wholly or partially identified. The term "lactic" in the table refers to organisms which produced a small acid colony on the litmus lactose agar plates. When some of these were transplanted to litmus milk they produced the typical lactic acid curd accompanied by a reduction of the litmus they all are, therefore, in all probability true members of the species, *Bacterium lactis acidi* or *Streptococcus lacticus*.

Inspection of the results in table 1 fails to reveal any very definite relationship between score and the number or kinds of bacteria or between the development of the particular off-flavors and the bacterial flora present.

Most of the raw cream butters had developed the old cream flavor at the end of nine days when the first scorings were made while the pasteurized cream butters had developed a metallic flavor. The old cream flavor usually was followed with increasing age by fishy flavor while the metallic flavor was not. This suggests that pasteurization may have had some effect in preventing this particular flavor, which in turn supports the idea suggested by Supplee (2) that a biological agent is concerned in the development of this flavor.

The development of the metallic flavor on the other hand seems to have been associated in these butters more with the pasteurized cream butters than with those made from raw cream. Possibly this was due to the use of a pasteurizer the surfaces of which had copper exposed and the combination of acid and high heat favored the formation of copper salts. In this case, however, one would expect the development, later, of tallowy flavor as shown by the results of Hunziker and Hosman (3) and those of Palmer and Combs (4). This, however, is not the case. The tallowy flavor developed much more frequently in the raw than in the pasteurized samples. The other flavors reported, occurred so infrequently that no definite conclusions can be drawn from them. It should be noted, however, that the acrid flavor developed much more frequently in the well ripened butters and in these latter more frequently in the pasteurized samples than in the raw samples. No reason has been assigned for this peculiarity.

Bacteriological findings. The most striking results of the bacteriological work were the following: (1) The relatively high counts of the butters when over a year old. There is a sharp falling off in numbers during the first nine days, after which the numbers gradually decline but leave higher counts than are usually reported for old butters.

(2) The relatively prolonged vitality of the lactic flora. In pure cultures in milk, which is usually considered the best medium for the growth of this species, the lactics usually die off within a week. In these butters, however, we find them persisting for as long as 275 days (and in one case for 426 days—sample 22).

TABLE 1

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 1 and 11 (duplicate). Raw cream (53°) churned at once [Control]						
days Fresh					20,500,000	Lactic, non-liquefying yeast, *Oidium
9	92	91	Old cream, unclean	Old cream	2,300,000	Lactic, *Oidium
48	88¾	88¾	Old	Old cream	2,800,000	Lactic, *liquefying yeast
164	88	88	Old cream, fishy, tallowy	Old cream	2,100,000	Lactic, *B. subtilis, non-liquefying yeast
275	88	87½	Old cream, fishy, tallowy	Old cream, strong, dish-rag	1,750,000	Lactic, Bact. lactis synxanthum, M. lactis roseceus
426	86½	85½	Fishy, tallowy	Unclean, metallic	1,900,000	*Liquefying yeast
Samples 2 and 12 (duplicate). Raw cream (53°) churned at once, butter washed with lactic acid						
9	91	93½	Old cream	Old cream	1,550,000	Lactic, *liquefying yeast, *Oidium
48	88	88	Old, unclean	Old cream, unclean	2,000,000	Lactic, *B. subtilis, *B. lactis tetragenus, *Oidium
164	89	85	Old cream	Fishy, rancid, moldy, unclean	1,800,000	Lactic, *B. subtilis, *Liquefying yeast, *Oidium
275	88	87½	Old cream, metallic	Old cream, stale, metallic	910,000	Lactic, *M. lactis aureus, *liquefying yeast
426	87	85½	Tallowy	Unclean, metallic	1,900,000	*Liquefying yeast

Samples 3 and 13 (duplicate). Raw cream (53°) churned at once, casein added						
9	87	91	Old cream, moldy	Old cream	3,500,000	Lactic, *Oidium, non-liquefying yeast, *liquefying yeast
48	89	90½	Old cream	Old cream	4,000,000	Lactic, *Oidium, *liquefying yeast
164	88½	85	Old cream	Fishy, rancid, moldy, unclean	2,600,000	Non-liquefying yeast
275	86½	85	Old cream, tallowy, metallic	Stale, dishrag, flat, metallic	2,750,000	Lactic, *liquefying yeast, non-liquefying yeast
426	87	87	Tallowy	Fishy, tallowy	690,000	*Liquefying yeast
Samples 4 and 14 (duplicate). Raw cream (53°) churned at once, fishy butter added						
9	93	91	Old cream	Old cream	2,050,000	Lactic, *Oidium, *Cladothrix, *liquefying yeast
48	88½	89½	Old cream	Old cream, abnormal acid	5,000,000	Lactic, *Oidium, *liquefying yeast
164	89	85½	Old cream, fishy	Old cream, fishy	3,300,000	*Liquefying yeast, *B. subtilis, M. lactis citreus B. (var. no pell.)
275	88	86	Old cream, tallowy	Old cream, fishy, tallowy	1,100,000	Lactic
426	87½	87	Tallowy	Fishy, tallowy	540,000	

* Organisms which liquefy either gelatine or milk or both.

TABLE 1—*Continued*

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 5 and 15 (duplicate). Raw cream (53°) churned at once, boric acid added						
days						
9	91	91	Old cream	Old cream, weak flavor	7,750,000	Lactic, *Oidium, *liquefying and non-liquefying yeasts
48	88	88½	Old cream	Old, flat, cheesy	5,000,000	Lactic, *Cladothrix, Streptococcus lactis aureus
164	89	87	Old cream	Old cream, sickish, feverish, light salt	2,800,000	Lactic, *B. subtilis, non-liquefying yeast
275	86½	86½	Old cream, fishy	Old cream, unclean	1,300,000	Lactic, *M. lactis albus, var. D.
426	87½	87	Fishy	Tallowy	1,150,000	
Samples 6 and 16 (duplicate). Pasteurized, not ripened, churned at once, [control]						
Fresh					155,000	
9	89	87	Metallic	Metallic, sickish	77,000	*Bact. lactis Marshalli, var. B.
48	88	88	Old cream, metallic	Old cream, metallic	32,000	*B. mycoides, Bact. lactis salmonis, *B. subtilis
164	87	88	Old cream, metallic	Old cream	45,000	*B. subtilis
275	84½	86½	Old cream, fishy, unclean, metallic	Strong, acrid, metallic	17,000	Non-liquefying yeast, *B. lactis Harrisoni (var.)
426	85	86	Metallic	Metallic	25,000	*Cladothrix, *Ps. lactis Eurotas

Samples 7 and 17 (duplicate). Pasteurized, not ripened, acidified H_2O in second washing						
9	87	91	Metallic	Metallic	51,000	*B. lactis Harrisoni (var.)
48	87¼	88⅔	Old cream	Old cream, metallic	22,500	*M. lactis aureus, *Oidium, non-liquefying yeast, Galactococcus versicolor (var.), *Bact. lactis fluorescens
164	88	86½	Old cream, unclean, metallic	Old cream, acrid, gritty	47,000	Galact. versicolor, lactic, *B. subtilis
275	87	87	Old cream, metallic	Strong, fishy, acrid, metallic	16,500	Lactics, Galact. versicolor
426	86½	86	Tallowy, metallic	Metallic	10,000	
Samples 8 and 18 (duplicate). Pasteurized cream, not ripened, casein added						
9	87	89	Metallic		85,000	*Bact. lactis Marshalli, var. B.
48	88½	89½	Old cream, metallic	Old	57,000	Galact. versicolor, *Streptococcus lactis citreus I.
164	88	87	Old cream, insipid, mealy, metallic	Old cream, dishrag, moldy, metallic	38,000	Lactics (and others)
275	87½	86	Old cream, strong, acrid, granular, mealy, metallic	Old cream, insipid, acrid, mineral flavor	15,500	Lactics, *Ps. lactis Eurotas, Strep. lactis citreus I. var. A.
426	86	86	Metallic	Tallowy, unclean, metallic	27,000	B. lactis nebulus

* Organisms which liquefy either gelatine or milk or both.

TABLE 1—*Continued*

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 9 and 19 (duplicate). Pasteurized cream, not ripened, fishy butter added						
days						
9	88	89	Old cream, metallic	Metallic	80,000	
48	88	89½	Old cream, metallic	Old cream, metallic	39,000	*Oidium, Bact. lactis salmonis, Galact. versicolor, *liquefying yeast, *Bact. lactis fluorescens
164	87	86½	Old cream, strong, metallic, abnormal acid	Pasty, unclean, metallic	82,000	Lactic (?), non-liquefying yeast
275	87	87½	Old cream, strong, metallic, heavy salt	Metallic, heavy salt	15,000	Lactics, Bact. lactis Marshalli, var. B.
426	85½	85½	Metallic		27,000	Non-liquefying yeast, *Ps. lactis Eurotas, *Ps. lactis Eurotas (var. no pellicle), *Ps. lactis Eurotas (rapid liquefying)
Samples 10 and 20 (duplicate). Pasteurized cream, not ripened, boric acid added						
9	88	89	Old cream, metallic	Metallic	60,000	Bact. lactis Marshalli, var. B., B. lactis Colchesterii
48	88	88½	Old cream, metallic, heavy salt	Old cream, rancid, metallic	40,000	Bact. lactis Marshalli, var. B.
164	88	86½	Old cream, metallic, gritty	Old cream, moldy unclean, metallic	32,000	Bact. lactis Marshalli, var. B.
275	87	88	Old cream, strong, metallic	Old cream, strong, metallic	20,500	Bact. lactis Marshalli, var. B., *M. lactis varians
426	86½	87	Metallic	Tallowy	29,000	*Ps. lactis Eurotas

Samples 21 and 31 (duplicate). Raw cream (53°) ripened to 60° acid [control]						
Fresh					25,500,000	Lactic, *liquefying yeast.
9	90	88	Old cream, acrid	Old cream, unclean	10,500,000	Lactic, *Oidium, *liquefying yeast
48	88	88½	Old, unclean, acrid, metallic	Old cream, old, metallic	2,900,000	Lactic, non-liquefying yeast
164	86½	85	Fishy, acrid, metallic	Fishy, unclean	830,000	Lactic, *liquefying yeast, B. lactis citreus, B. lactis varians (var.)
275	86	85	Old cream, acrid, metallic	Strong, fishy, metallic	850,000	Lactic (?), *liquefying yeast
426	86½	85	Fishy	Fishy, unclean	100,000	Lactic
Samples 22 and 32 (duplicate). Raw cream (53° acid), ripened to 60° acid, washed in acidified water						
9	89	87	Cheesy, acrid	Old cream, unclean	9,050,000	Lactic, *Oidium, *liquefying yeast, M. lactis rosaceus (var.)
48	86½	87½	Old, fishy	Foreign, abnormal acid, metallic, heavy salt	4,600,000	Lactic, *Oidium, *liquefying yeast, *B. subtilis
164	86½	86½	Acrid, gritty, metallic	Acrid, gritty	2,500,000	Lactic, *liquefying yeast
275	87	89	Old cream, tallowy, acrid, metallic	Old cream, good flavor	1,600,000	Lactic (?), *liquefying yeast, Galact. versicolor
426	86½	86		Acrid, metallic	1,000,000	Lactics

* Organisms which liquefy either gelatine or milk or both.

TABLE 1—*Continued*

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 23 and 33 (duplicate). Raw cream (53° acid), ripened to 60° acid, casein added						
days						
9	91	89	Old cream	Old cream, tallowy, unclean	8,800,000	Lactic, *Oidium, *liquefying yeast
48	89	88½	Old	Old cream, metallic	4,250,000	Lactic, *B. subtilis, Bact. lactis cretaceum
164	85	88½	Old cream, fishy, unclean, metallic	Old cream, fishy	2,500,000	Lactic, *liquefying yeast, non-liquefying yeast, Bact. lactis cretaceum
275	86	86½	Fishy, acrid, mealy, mineral flavor	Fishy, tallowy, metallic	2,550,000	Galact. versicolor
426	87	85½	Fishy	Fishy, metallic	1,827,000	*Liquefying yeast
Samples 24 and 34 (duplicate). Raw cream (53° acid), ripened to 60° acid, fishy butter added						
9	92	87	Old cream, tallowy	Old cream, old, stale	3,650,000	Lactic, *Oidium
48	89½	88½	Old cream, metallic	Old cream, old, unclean	3,250,000	Lactic, *Oidium, *Bact. lactis fluorescens, M. lactis citreus B (var. no pell.), S. lactis citreus I. (var. A.)
164	85	87	Old cream, fishy, unclean, metallic	Fishy	480,000	Lactic, *Oidium
275	85	89	Old cream, strong, fishy	Old cream, good flavor	170,000	Lactic
426	87	86½	Fishy	Fishy, metallic	1,047,000	Non-liquefying yeast

Samples 25 and 35 (duplicate). Raw cream (53°+), ripened to (60°+), boric acid added						
9	92	92	Old cream	Old cream	7,900,000	Non-liquefying yeast, *Oidium
48	90⅞	88⅞	Old cream, good flavor, acrid	Weak flavor, unclean	4,050,000	Lactic, *Oidium, *liquefying yeast, *M. lactis varians, M. lactis varians A. (var.)
164	85	88	Old cream, fishy, moldy, metallic, unclean	Old cream, fishy	2,500,000	Lactic, *liquefying yeast, B. lactis citreus
275	84	86	Old cream, strong, fishy	Strong, fishy, flat	730,000	Lactic
426	85½	86½	Fishy	Fishy, metallic, light salt	1,300,000	
Samples 26 and 36 (duplicate). Pasteurized cream (53° acid), ripened to 64°+ [control]						
Fresh					18,000,000	Lactics
9	87	89	Metallic	Metallic	1,900,000	Small white lactic
48	87	87	Old, unclean, metallic	Dishrag, metallic	410,000	Lactic
164	86½	85½	Old cream, metallic	Old, woody, moldy	470,000	Lactic
275	86½	85½	Old cream, flat, metallic	Old cream strong, fishy, acrid, mealy, metallic	78,000	Lactic, Bact. lactis Marshalli, var. B.
426	85½	86	Metallic	Fishy, metallic	330,000	
Samples 27 and 37 (duplicate). Pasteurized cream, ripened to 64°, washed in acidified H_2O						
9	90	92	Old cream, acrid, metallic	Old cream, metallic	640,000	Lactics
48	86⅞	86⅞	Old, unclean	Abnormal acid, metallic	470,000	Lactics
164	87½	87½	Old cream, acrid, metallic	Old cream, metallic	1,900,000	Lactics, non-liquefying yeast
275	87½	86	Old cream, strong, acrid	Fishy, flat, acrid, mealy	230,000	Non-liquefying yeast
426	86	87	Acrid, metallic	Metallic	320,000	

* Organisms which liquefy either gelatine or milk or both.

TABLE 1—*Concluded*

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 28 and 38 (duplicate). Pasteurized cream, ripened to 64°, casein added						
days						
9	87	89	Tallowy, metallic	Metallic	700,000	Lactic
48	88	88	Old cream, metallic	Old cream, metallic	270,000	Lactics
164	86½	87	Acrid, metallic	Strong, acrid	330,000	Lactics
275	86	86½	Strong, acrid, metallic	Strong, fishy, acrid, metallic	86,000	Lactics, M. lactis citreus (var., no pell.)
426	85	87	Metallic	Metallic	273,000	
Samples 29 and 39 (duplicate). Pasteurized cream, ripened to 64°+, fishy butter added						
9	88	89	Old cream, metallic	Old cream, metallic	700,000	Lactic
48	88½	89	Old, fishy, tallowy, unclean	Old cream, metallic	210,000	Lactic
164	86½	86½	Acrid, metallic	Unclean, acrid, metallic	31,000	Lactic
275	86½	86½	Old cream, flat, acrid, metallic	Strong, acrid	28,000	Bact. lactis Marshalli, var. B.
426	85½	86	Metallic	Metallic	167,000	*Ps. lactis Eurotas
Samples 30 and 40 (duplicate). Pasteurized cream, ripened to 64°+, boric acid added						
9	87	90	Tallowy, metallic	Metallic	540,000	Lactic
48	88½	88	Old cream, metallic	Old cream, abnormal acid, metallic	210,000	Lactic, *Oidium
164	85½	86½	Acrid, metallic	Unclean, acrid	200,000	Lactic
275	87	86½	Old, flat, tallowy, acrid	Metallic	280,000	Lacti. *M lactis varians
426	86½	87	Strong, metallic		112,000	

* Organisms which liquefy either gelatine or milk or both.

Only two possible reasons have suggested themselves to the writers. One is that possibly the association with other bacteria may in some way prolong their activity. The other is that the fat may exert a protective influence by shielding certain favorably placed cells (those in the fat itself, not in the curd) from the acid produced by the cells. The low temperature and the salt too may cause such a slow rate of metabolism that relatively little acid is produced and so the life of the cell prolonged.

The most frequent species observed aside from the lactics were a liquefying and a non-liquefying yeast and a species of Oidium. Both the liquefying yeast and the Oidium occur less frequently (in fact, only rarely) in the pasteurized cream butters than in the raw cream butters. This suggests that these organisms entered the cream largely before pasteurization and were killed off during the process. The few that occurred in the pasteurized cream butters may have come from unsterile butter utensils or from the air.

Every one of the butters except samples 26, 27, and 28 contained one or more and in some cases as high as six different species of liquefying organisms. These are indicated in the table by an asterisk. The presence of these organisms helps to explain the production of the soluble nitrogen noted below.

The salt agar plates incubated at room temperature contained relatively few bacteria, the counts being 9500 per gram, 2800 per gram, 8100 per gram, and 3100 per gram for samples I, VI, XXI, and XXVI, the butters which were examined when freshly made. At nine days the highest count obtained was 6300 per gram for sample IX. The numbers usually were below 3000 per gram for the other samples, with only a few hundred remaining at the end of the period of storage.

The salt agar plates incubated at 6°C. made a much poorer showing than those incubated at room temperature, the counts ranging from 0° to 2200 per gram.

The flora isolated from the salt agar plates contained practically the same predominant forms as appeared on the litmus lactose agar plates except that the lactics were completely repressed by the salt. A number of species not appearing on the

litmus lactose agar plates appeared on the salt agar plates but only in small numbers so that they are of no particular significance.

ANALYTICAL DATA

The analytical data were secured in the hope that by their aid some of the changes taking place in the butter might be explained. Previous papers from this station had suggested that some of the off flavors were due to changes in the protein portion of the butter (5).

TABLE 2

Per cent of moisture and of salt in the experimental butters

NUMBER OF SAMPLE	MOISTURE				SALT					SALT IN MOISTURE
	Age of butter (days)			Average	Age of butter (days)				Average	
	11	50	166		11	50	166	277		
										per cent
1	13.95	13.74	14.09	13.93	2.40	2.45	2.50	2.27	2.51	18.0
2	13.27	13.54	13.12	13.31	3.55	3.60	3.55	4.00	3.65	27.4
3	14.03	14.00	14.39	14.14	2.40	2.40	2.45	2.20	2.36	16.7
4	14.07	13.95	14.08	14.03	2.40	2.50	2.45	2.55	2.47	17.6
5	14.20	14.37	14.23	14.27	2.55	2.50	2.60	2.65	2.57	18.0
6	18.75	19.10	18.88	18.91	4.55	4.50	4.65	4.75	4.61	24.3
7	14.54	14.38	13.54	14.49	4.20	4.15	3.60	3.90	3.96	27.4
8	19.83	19.70	19.82	19.78	4.85	4.90	4.95	5.05	4.94	25.0
9	19.45	19.21	19.16	19.27	4.65	4.80	4.70	4.90	4.74	24.6
10	19.62	19.60	19.32	19.51	4.75	4.85	4.95	4.90	4.86	24.9
21	16.31	16.12	16.40	16.28	3.35	3.40	3.35	3.20	3.32	20.4
22	13.82	13.95	14.03	13.93	4.25	4.30	4.00	4.20	4.19	30.0
23	16.26	16.13	16.37	16.25	3.30	3.40	3.35	3.50	3.39	20.8
24	16.10	16.17	16.08	16.12	3.30	3.25	3.10	3.20	3.21	19.9
25	16.15	16.62	16.26	16.34	3.35	3.40	3.25	3.40	3.35	20.5
26	19.53	19.06	19.68	19.42	4.80	4.70	4.60	4.55	4.66	24.0
27	14.58	14.65	15.11	14.81	3.80	3.65	3.75	3.50	3.67	24.8
28	19.93	19.86	19.81	19.87	4.75	4.80	4.85	5.10	4.78	24.5
29	19.52	19.72	19.44	19.56	4.65	4.70	4.60	4.60	4.64	23.7
30	19.94	19.89	19.43	19.74	4.75	4.80	4.55	4.60	4.67	23.6

Salt and moisture. The salt and moisture determinations are recorded in table 2.

It will be seen that a number of the samples contained a high percentage of moisture this being especially true of the pasteurized samples. The same thing may be said of these samples in respect to the percentage of salt. In the last column are the calculated percentages of the strengths of brine in each butter.

TABLE 3

Lactose in the experimental butters

NUMBER OF SAMPLE	PER CENT IN BUTTER					AS PER CENT OF MOISTURE IN BUTTER				
	Age of butter (days)					Age of butter (days)				
	At first	11	166	277	428	At first	11	166	277	428
1	0.30	0.29	0.28	0.27	0.27	2.16	2.08	2.02	1.94	1.94
2		0.28	0.29	0.28	0.28		2.14	2.21	2.12	2.12
3		0.31	0.29	0.29	0.29		2.18	2.05	2.05	2.05
4		0.30	0.28	0.28	0.28		2.14	2.00	2.00	2.00
5		0.30	0.29	0.29	0.28		2.10	2.02	2.02	1.96
6	0.32	0.29	0.27	0.26	0.26	1.70	1.54	1.43	1.38	1.38
7		0.33	0.32	0.28	0.28		2.32	2.25	1.97	1.97
8		0.28	0.26	0.27	0.26		1.41	1.31	1.36	1.31
9		lost	0.33	0.32	0.30		lost	1.71	1.65	1.56
10		0.31	0.27	0.30	0.29		1.59	1.39	1.54	1.49
21	0.33	0.29	0.28	0.29	0.29	2.02	1.78	1.72	1.78	1.78
22		0.30	0.29	0.30	0.29		2.16	2.08	2.16	2.18
23		0.33	0.32	0.31	0.31		2.02	1.91	1.91	1.91
24		0.30	0.29	0.30	0.30		1.87	1.81	1.87	1.87
25		0.33	0.31	0.31	0.31		2.02	1.90	1.90	1.90
26	0.31	0.30	0.28	0.28	0.28	1.60	1.54	1.45	1.45	1.45
27		0.29	0.29	0.27	0.28		1.96	1.96	1.83	1.89
28		0.30	0.29	0.29	0.29		1.51	1.46	1.46	1.46
29		0.29	lost	0.29	0.29		1.48	lost	1.48	1.48
30		0.29	0.28	0.29	0.28		1.47	1.42	1.47	1.42
Average	0.315				0.2855	1.87				1.756

This makes the high bacteriological counts already discussed all the more striking since most of these results show very strong brines, some of them being about two-thirds saturated solutions.

Lactose. In table 3 are shown the percentages of lactose in the butters and a recalculation of the results in terms of percentage of lactose in the moisture of the butter.

If the average of the amount of lactose in all of the butters when fresh is compared with the average at the age of 426 days it will be seen that there is a slight but unmistakable decline in the amount of lactose. The average when fresh is 0.315 per cent and when old is 0.2855 per cent, or in terms of the moisture

TABLE 4

Acidity determinations in experimental butters (cubic centimeters of N/10 NaOH per 100 grams butter)

NUMBER OF SAMPLE	ACIDITY OF BUTTER						ACIDITY WASHED FROM BUTTER					
	Age of butter (days)						Age of butter (days)					
	At first	11	50	166	277	428	At first	11	50	166	277	428
1	25	27	28	31	30	34	3.0	4.0	3.5	3.4	3.3	3.3
2		32	30	34	30	32		5.3	3.2	4.3	3.8	4.0
3		29	29	34	32	36		4.8	3.6	4.0	3.9	3.5
4		28	29	32	30	33		4.9	3.6	3.8	3.7	3.7
5		30*	24*	25*	28*	23*		7.0*	6.8*	4.4*	4.5*	3.8*
		(50)	(44)	(45)	(48)	(43)		(9.0)	(8.8)	(6.4)	(6.5)	(5.8)
6	18	20	17	20	21	20	2.1	2.7	2.4	2.1	1.8	2.0
7		22	21	22	24	22		3.0	2.6	2.9	3.1	3.1
8		20	19	19	20	20		2.7	2.5	2.5	2.6	2.6
9		18	18	19	20	18		2.6	2.5	2.7	2.1	2.1
10		20*	17*	23*	25*	27*		6.7*	5.7*	3.9*	4.0*	3.5*
		(35)	(32)	(38)	(40)	(42)		(8.2)	(7.2)	(5.4)	(5.5)	(5.0)
21	26	32	30	33	31	30	4.0	4.3	4.3	4.9	4.1	3.7
22		32	31	36	32	38		4.6	4.3	4.3	3.2	3.2
23		32	28	34	32	30		5.0	4.8	4.5	4.5	4.1
24		32	32	38	37	38		5.7	6.1	5.5	4.5	5.7
25		32*	30*	33*	32*	31*		6.4*	6.2*	5.6*	6.4*	7.2*
		(45)	(43)	(46)	(45)	(44)		(7.7)	(7.5)	(6.9)	(7.7)	(8.5)
26	20	20	20	21	20	20	2.4	3.1	2.4	3.0	2.7	3.2
27		21	23	24	24	22		3.7	2.3	3.5	3.1	2.4
28		20	20	22	25	24		3.8	2.2	3.2	3.0	2.2
29		21	22	23	24	22		4.1	3.6	3.0	2.9	2.5
30		31*	24*	26*	22*	24*		7.4*	5.6*	3.7*	3.4*	3.2*
		(49)	(42)	(44)	(40)	(42)		(9.2)	(7.4)	(5.5)	(5.2)	(5.0)
Average	322.5	27.2					32.875	3.95				

* Acidity due to boric acid added is not included.

in the butter, the figures become 1.87 and 1.756 per cent, respectively. This, no doubt, was used up in producing acid. The smallness of the amount used (viz., 0.095 per cent) for the production of acid supports the theory expressed on a previous page that the metabolism of the acid forming bacteria was retarded by the presence of the salt and the low temperature of storage.

Acidity. The determinations of the acidity of the butters are presented in table 4. As is to be expected, the acidity increased during the period of storage. The average number of cc. of N/10 soda per 100 grams of butters when fresh was 22.5, and after 426 days of storage was 27.2, a difference of 4.7 cc. Expressed in terms of acid washed from butter, representing soluble acid, the figures become 2.875 cc. for the average of the fresh butters, and 3.95 cc. for the average of the old butters, a difference of 1.075 cc. This is not very much but it is many times the amount that could have come from the average amount of lactose used up. This suggests that there was some hydrolysis of the fat or protein resulting in compounds capable of using up alkali. No other explanation except the unwelcome one of errors in technique suggests itself.

DEGRADATION OF THE NITROGEN

The total nitrogen for the various butters at the age of 11 and 166 days are given in table 5. The first three columns give the results calculated as per cent of the butter and the last two columns, as per cent of the moisture in the butter.

The most striking thing about these results is the fact that the pasteurized samples contained about one half of the nitrogen that was contained in the raw butters. These are similar to the results obtained by Mortensen, Gaessler, and Cooper (6), who found that when the cream was pasteurized in the sour condition, the per cent protein of the resulting butter was decreased over raw cream butter but that when sweet cream was pasteurized the protein content of the butter was not influenced. They offer the explanation that in sour cream "the casein in the presence

of acid is hardened and thrown into clumps known as curd particles, that are quite readily removed in the cleaning and washing of the butter." Hunziker, Spitzer, Mills and Switzer (7) also note a tendency of pasteurization to lower the curd in butter,

TABLE 5

Total nitrogen in experimental butters

NUMBER OF SAMPLE	AS PER CENT IN BUTTER		AS PER CENT OF MOISTURE IN BUTTER	
	Age of butter (days)		Age of butter (days)	
	11	166	11	166
1	0.0756	0.0756	0.582	0.582
2	0.0812	0.0770	0.614	0.588
3	0.0850* (0.1090)	0.0740* (0.0980)	0.600* (0.769)	0.522* (0.683)
4	0.0812	0.0792	0.580	0.565
5	0.0798	0.0826	0.556	0.578
6	0.0518	0.0490	0.274	0.259
7	0.0504	0.0490	0.355	0.346
8	0.0478* (0.0756)	0.0492* (0.0770)	0.242* (0.381)	0.248* (0.389)
9	0.0476	0.0462	0.247	0.239
10	0.0476	0.0462	0.245	0.237
21	0.0868	0.0854	0.531	0.524
22	0.0840	0.0770	0.605	0.555
23	0.0842* (0.1120)	0.0830* (0.1108)	0.518* (0.699)	0.509* (0.685)
24	0.0854	0.0840	0.530	0.522
25	0.0868	0.0854	0.532	0.524
26	0.0560	0.0546	0.288	0.282
27	0.0588	0.0546	0.396	0.370
28	0.0537* (0.0924)	0.0523* (0.0910)	0.270* (0.465)	0.264* (0.457)
29	0.0532	0.0490	0.272	0.250
	0.0504	0.0476	0.256	0.242

but offer no explanation for the fact, though their explanation for loss of fat in the buttermilk would apply to the loss of curd as well; since they also postulate a shrinking of the curd in the presence of high acid and high heat.

Another explanation that may account for the lesser amounts of protein in the pasteurized cream butters is that the presence

of acid and heat may have resulted in a hydrolysis of a portion of the casein which would then be lost during the subsequent washing of the butters since it would then be soluble.

The nitrogen not precipitated by copper sulphate appears in table 6 where it is recorded both as percentage of the butter

TABLE 6

Nitrogen not precipitated by copper sulfate

NUMBER OF SAMPLE	AS PER CENT OF BUTTER						AS PER CENT OF MOISTURE IN BUTTER					
	Age of butter (days)						Age of butter (days)					
	At first	11	50	166	277	428	At first	11	50	166	277	428
1	0.0060	0.0064	0.0064	0.0068	0.0071	0.0064	0.0432	0.0461	0.0461	0.0488	0.0510	0.0461
2		0.0063	0.0063	0.0059	0.0067	0.0067		0.0481	0.0481	0.0454	0.0511	0.0511
3		0.0064	0.0064	0.0064	0.0071	0.0064		0.0450	0.0450	0.0450	0.0499	0.0486
4		0.0060	0.0060	0.0064	0.0071	0.0064		0.0429	0.0429	0.0457	0.0502	0.0457
5		0.0060	0.0060	0.0060	0.0071	0.0064		0.0420	0.0420	0.0420	0.0492	0.0447
6	0.0031	0.0031	0.0031	0.0027	0.0035	0.0038	0.0164	0.0164	0.0164	0.0144	0.0185	0.0202
7		0.0030	0.0030	0.0026	0.0030	0.0030		0.0211	0.0211	0.0183	0.0211	0.0211
8		0.0031	0.0035	0.0031	0.0035	0.0035		0.0157	0.0177	0.0157	0.0181	0.0159
9		0.0027	0.0031	0.0027	0.0035	0.0035		0.0140	0.0161	0.0140	0.0181	0.0181
10		0.0027	0.0031	0.0027	0.0031	0.0031		0.0139	0.0159	0.0139	0.0159	0.0159
21	0.0064	0.0068	0.0064	0.0064	0.0076	0.0076	0.0392	0.0392	0.0417	0.0392	0.0465	0.0465
22		0.0056	0.0060	0.0056	0.0064	0.0060		0.0403	0.0432	0.0403	0.0460	0.0432
23		0.0064	0.0068	0.0064	0.0076	0.0072		0.0392	0.0416	0.0392	0.0465	0.0441
24		0.0064	0.0057	0.0061	0.0072	0.0072		0.0398	0.0355	0.0377	0.0448	0.0448
25		0.0061	0.0061	0.0057	0.0076	0.0068		0.0374	0.0374	0.0350	0.0464	0.0418
26	0.0035	0.0038	0.0035	0.0035	0.0038	0.0035	0.0180	0.0196	0.0180	0.0180	0.0196	0.0180
27		0.0034	0.0038	0.0034	0.0030	0.0030		0.0123	0.0157	0.0123	0.0206	0.0206
28		0.0039	0.0039	0.0039	0.0039	0.0039		0.0196	0.0196	0.0196	0.0196	0.0196
29		0.0039	0.0039	0.0035	0.0039	0.0039		0.0199	0.0199	0.0179	0.0199	0.0199
30		0.0031	0.0035	0.0031	0.0031	0.0035		0.0158	0.0177	0.0158	0.0158	0.0177

and as percentage of the moisture in the butter. This data has also been recalculated and recorded in table 7 as percentage of the total nitrogen.

As shown by table 7 there are only four exceptions (samples 5. 7, 26, and 27) to the rule that there was a higher percentage of soluble nitrogen at the end of the period of storage than when

the butters were fresh. In a general way this decomposition was progressive though there were many exceptions to the rule. There are also some cases where there was an apparent decrease in the percentage of soluble nitrogen. This, no doubt, was due to errors in technique or variation in the distribution of the

TABLE 7

Nitrogen not precipitated by copper sulfate (recorded in per cent of total nitrogen)

NUMBER OF SAMPLE	AT FIRST	11 DAYS	50 DAYS	166 DAYS	277 DAYS	428 DAYS
1	7.95	8.47	8.47	8.99	9.39	8.47
2		7.96	7.96	7.45	8.46	8.46
3		8.05*	8.05*	8.05*	8.91*	8.55*
		(6.19)	(6.19)	(6.19)	(6.88)	(6.58)
4		7.48	7.48	7.98	8.85	7.98
5		7.39	7.39	7.39	8.74	7.88
6	6.15	6.15	6.15	5.36	6.94	7.54
7		6.04	6.04	5.23	6.04	6.04
8		6.40*	7.20*	6.40*	7.21*	7.21*
		(4.07)	(4.58)	(4.07)	(4.58)	(4.58)
9		5.75	6.61	5.75	7.46	7.46
10		5.75	6.61	5.75	6.61	6.61
21	7.43	7.43	7.89	7.43	8.81	8.81
22		6.95	7.46	6.95	7.95	7.46
23		7.58*	8.05*	7.58*	9.00*	8.51*
		(5.69)	(6.03)	(5.69)	(6.75)	(6.40)
24		7.55	6.73	7.20	8.50	8.50
25		7.00	7.00	6.55	8.27	7.82
26	6.34	6.87	6.34	6.34	6.87	6.34
27		6.00	6.71	6.00	5.29	5.29
28		7.36*	7.36*	7.36*	7.36*	7.36*
		(4.26)	(4.26)	(4.26)	(4.26)	(4.26)
29		7.64	7.64	6.85	7.64	7.64
30		6.33	7.14	6.33	6.33	7.14

* Nitrogen added to butter in form of casein is not included.

nitrogen compounds in the butters. The increase in soluble nitrogen as recorded in table 7 follows in a general way the decrease in total nitrogen as shown by table 5.

A curious fact may be noted in connection with these analyses. While the pasteurized cream butters contained only about one-

half of the amount of nitrogen as was contained in the raw cream butters, and the percentage of soluble nitrogen was also lower in the pasteurized butters than in the raw butters, the percentage of the total nitrogen which was rendered soluble was about the same in both types of butters. The explanation for this fact is not clear (though it probably could be explained by the law of mass action) since "it is known that protein may hydrolize on long standing, in the presence of water alone without the assistance of any intermediary agents" (8).

SUPPLEMENTARY WORK ON THE ACTION OF THE BUTTER FLORA ON MILK

Supplementary to the work outlined above some additional work was done with some of the typical butter micro-organisms to study their individual action upon milk. It is known that the micro-organisms most commonly found in butter are to a great extent those bacteria that were present in the milk and cream from which the butter was made; therefore, the initial butter flora is milk flora and vice versa. The changes taking place in milk can be more easily studied since they proceed with greater rapidity than the changes taking place in butter. Only the possibility of growth in milk and the action upon the nitrogenous constituents will be considered here.

The casein in butter during storage is slowly broken down into amino-acids and ammonia. Nitrogen, as amino-acids and ammonia, in percentage of the total nitrogen in unsalted butter (average of tubs from the creameries) was found in a previous study (9) to be 5.71 per cent at first and 7.59 per cent after 240 days in storage at 21°F.; in salted butter from the same three churnings and held in the same storage, it was found to be 5.71 per cent at first and 8.19 per cent after 240 days. And again as shown in the present study (see table 7) in salted butter made from pasteurized and from unpasteurized cream, the percentage (average of 20 tubs) increased from 6.24 per cent to 6.86 per cent for the pasteurized and from 7.68 per cent to 8.25 per cent for the unpasteurized butter during storage at 0°F. for 428 days.

Milk was found by M. E. Pennington (10) to contain certain species of bacteria especially resistant to cold; these became numerous and frequently occurred in almost pure culture after five to six weeks at 0.55°C. As a rule the liquefying group increased in proportion to the acid forming group. The casein of the milk in cold storage was rapidly digested until more than 50 per cent was changed into soluble compounds. Working with milk at low temperature Ravenel, Hastings and Hammer (11) give data showing that milk held at 0°C. for 203 days increased markedly in bacterial content which resulted in a rise in acidity to 0.70 per cent and a change in the casein rendering 70 per cent of the total nitrogen soluble. In milk held at 0°C., there was no increase in bacterial count, a slight decrease in acidity and 20 per cent of the total nitrogen was soluble after 203 days.

When the growth in plain milk of twelve different bacteria isolated from storage butter was compared with their growth in milk to which had been added 5 per cent sterile salt, it was observed (table 8) that while all of these bacteria were capable of making a good growth on agar containing 12 per cent salt—seven grew well on 16 per cent—the addition of 5 per cent salt to milk had a slight retarding effect. One of the organisms, however, (M. l. citronus) grew as well or even made a better growth in salted milk. The number added per cubic centimeter of milk taking the average for the twelve different organisms was 2.322 which increased in plain milk during one, three, and seven days at 20°C. to 140,000, 12,860,000 and 80,650,000, respectively, and in the milk plus 5 per cent salt to 113,800, 5,743,000 and 14,924,000, respectively. As none of these organisms form more than a trace of acid the acidity of the milk showed only a slight increase, the average acidity after one, three and seven days being 0.18 per cent, 0.20 per cent and 0.20 per cent for plain milk and 0.19 per cent, 0.21 per cent and 0.20 per cent for the milk plus 5 per cent salt.

The action exerted by these bacteria upon the casein was influenced by the presence of salt very much in the same way as the rate of growth was influenced, that is, where the rate of

TABLE 8

Bacterial count and acidity

ORGANISM	AT FIRST		1 DAY		3 DAYS		7 DAYS	
	Acid	Count	Acid	Count	Acid	Count	Acid	Count
Milk without salt at 20°C.								
	per cent		*per cent*		*per cent*		*per cent*	
B. lactis Harrisoni..	0.10	10	0.15	10,000	0.22	3,000,000	0.32	1,160,000
Bact. lactis cretaceum.....	0.16	3,000	0.15	100,000	0.17	75,000,000	0.07	120,000,000
B. subtilis.....	0.16	1,185	0.17	10,000	0.17	2,250,000	0.17	17,000,000
B. mycoides.....	0.16	49	0.19		0.21	3,000,000	0.26	1,000,000
S. lactis citreus 1...	0.16	17,500	0.18	833,000	0.18	14,450,000	0.15	162,000,000
M. lactis varians A.	0.16	26	0.18	50,000	0.18	1,000,000	0.20	
(?).....	0.16	1	0.17	5,000	0.19		0.19	
M. lactis albus var. D.....	0.18	410	0.20	100,000	0.19		0.29	414,000,000
Ps. lactis Eurotas..	0.18	685	0.20	250,000	0.21	950,000	0.22	7,250,000
M. lactis rubidus...	0.18	30	0.20		0.20			
M. lactis citronus...	0.18	4,310	0.20	41,500	0.20	4,260,000	0.22	3,350,000
B. (?).....	0.18	675	0.21	1,000	0.22	40,000		120,000
Average.....	0.17	2,322	0.18	140,000	0.20	12,860,000	0.20	80,650,000
Milk with 5 per cent salt at 20°C.								
B. lactis Harrisoni..	0.16	10	0.14	1,000	0.20	100,000	0.17	580,000
Bact. lactis cretaceum.....	0.16	3,000	0.14	100,000	0.19	30,000,000	0.16	26,000,000
B. subtilis.....	0.16	1,185	0.18	10,000	0.19	1,400,000	0.21	23,000,000
B. mycoides.....	0.16	49	0.19	1,000	0.23	550,000	0.25	2,000,000
S. lactis citreus 1...	0.16	17,500	0.19	735,000	0.20	9,400,000	0.17	41,000,000
M. lactis varians A.	0.16	26	0.20		0.20		0.20	
(?).....	0.16	1	0.18	5,000	0.20		0.19	
M. lactis albus var. D.....	0.18	410	0.20	30,000	0.19	130,000	0.21	320,000
Ps. lactis Eurotas..	0.18	685	0.20	150,000	0.22	685,000	0.22	2,050,000
M. lactis citronus...	0.18	30	0.21	105,000	0.24	9,500,000	0.21	227,540,000
M. lactis rubidus...	0.18	4,310	0.20		0.22		0.20	
B. (?).....	0.18	675	0.22	1,000	0.24	20,000	0.24	
Average.....	0.17	2,322	0.19	113,800	0.21	5,743,000	0.20	14,924,000

growth was greatest the action was most marked (see tables 9 and 10). This group of twelve bacteria is capable of transforming casein into caseoses and caseones and then into amino-acids and ammonia, although with some of the organisms it appears from

TABLE 9

Nitrogen as caseoses and caseones (per cent of total milk)

ORGANISM (AT 20°C.)	MILK WITHOUT SALT			MILK WITH 5 PER CENT SALT		
	1 day	3 days	7 days	1 day	3 days	7 days
B. lactis Harrisoni	0.023	0.072	0.087	0.028	0.029	0.069
Bact. lactis cretaceum	0.023	0.028	0.096	0.024	0.029	0.046
B. subtilis	0.027	0.028	0.040	0.026	0.028	0.029
B. mycoides	0.032	0.046	0.048	0.031	0.033	0.050
S. lactis citreus 1	0.029	0.031	0.042	0.028	0.029	0.036
M. lactis varians A	0.026	0.032	0.036	0.028	0.034	0.034
(?)	0.029	0.029	0.069	0.034	0.034	0.034
M. lactis albus var. D		0.030	0.037		0.035	0.034
Ps. lactis Eurotas	0.035	0.035	0.036	0.035	0.038	0.038
M. lactis rubidus	0.036	0.037	0.042	0.038	0.039	0.046
M. lactis citronus	0.035	0.035	0.074	0.038	0.039	0.044
B. (?)	0.043	0.042	0.046	0.042	0.042	0.046
Average	0.031	0.037	0.054	0.030	0.034	0.042

TABLE 10

Nitrogen as amino-acids and ammonia (per cent of total milk)

ORGANISM (AT 20°C.)	MILK WITHOUT SALT			MILK WITH 5 PER CENT SALT		
	1 day	3 days	7 days	1 day	3 days	7 days
B. lactis Harrisoni	0.022	0.072	0.200	0.018	0.024	0.024
Bact. lactis cretaceum	0.023	0.026	0.107	0.019	0.023	0.025
B. subtilis	0.028		0.041	0.022	0.026	0.023
B. mycoides	0.031	0.072	0.130	0.029	0.043	0.082
S. lactis citreus 1	0.029	0.031	0.054	0.025	0.028	0.042
M. lactis varians A	0.027	0.034	0.034	0.030	0.035	0.035
(?)	0.026	0.034	0.106	0.027	0.031	0.033
M. lactis albus var. D	0.037	0.034	0.038	0.029	0.034	0.036
Ps. lactis Eurotas	0.037		0.038	0.035	0.033	0.041
M. lactis rubidus	0.037	0.038	0.039		0.038	0.046
M. lactis citronus	0.037		0.079	0.037	0.036	0.043
B. (?)	0.042	0.042	0.050	0.040	0.042	0.045
Average	0.031	0.042	0.076	0.028	0.035	0.041

our few analyses that amino-acids and ammonia increase as soon as the action upon the casein begins. Products of the first stages of casein decomposition were found after one, three, and seven days in the following amounts (average for the twelve bacteria) 0.031 per cent, 0.037 per cent and 0.054 per cent for plain milk and 0.030 per cent, 0.034 per cent and 0.042 per cent for the milk with 5 per cent salt. Amino acids and ammonia appeared as follows 0.031 per cent, 0.042 per cent, and 0.076 per cent for plain milk and 0.028 per cent, 0.035 per cent and 0.041 per cent salted milk. If plotted the curves for the changes in salted milk would be found to be the same in nature as those for the plain milk but of a lower magnitude.

CONCLUSIONS

While the results of this study failed to reveal any striking proofs of the relationship between methods of manufacture and either the score or the development of definite off-flavors, yet certain facts stand out sufficiently well to warrant mention. They are as follows:

The raw cream butters quickly developed the old cream flavor which was later followed by fishy flavor, while the pasteurized (sour) cream butters early developed a metallic flavor. Tallowy flavor developed more frequently in the raw cream butters than in the pasteurized cream butters. Acrid flavor developed much more frequently in well ripened pasteurized butters than in raw butters. From this study there is no evidence that either pasteurization or ripening improves the keeping quality of butters made from cream which has already soured.

Relatively higher bacteriological counts were obtained on butters over a year old than are usually obtained in studies of this kind, though there was a fairly rapid dying off in members at first.

The lactic acid bacteria appeared on the plates for a longer period than is usually thought possible, though there was a gradual displacement of the lactic acid flora by a more miscellaneous flora, among which the predominant types were a liquefying and a non-liquefying yeast and an oidium species.

Taking the butters as a whole, there was a slow gradual decrease in the amount of lactose present and a gradual increase in acidity, though the decrease of the one was not proportional to the increase in the other.

The amount of nitrogen in the pasteurized cream butters was about half that of the raw cream butters, but the percentage of nitrogen in soluble form was about the same in each class of butters, though it was very small in either case.

When 12 of the typical butter organisms were inoculated into milk alone and milk containing 5 per cent of salt, it was found that the number of bacteria increased rapidly in both cases but more rapidly when salt was not present. The increase in acidity was very slight in either case and of about the same amount. The action of these organisms upon the nitrogenous compounds of milk without and with the addition of 5 per cent salt was to increase the amount of soluble nitrogen in both cases but more so without than with salt. This amount of salt, however, does not retard the growth and action of these butter organisms as much as might seem possible.

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AN ASSOCIATIVE STUDY OF STREPT. LACTICUS AND B. SUBTILIS IN MILK

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There is well-known evidence in the literature (1) of bacteriology that concerns the growth of several microörganisms together in the same medium. In such associative growth the development of each organism is likely to be influenced by the development of the others; the life curve of each will probably vary from the natural life curve of the organisms in pure culture. Since milk ordinarily contains a comparatively large number of microörganisms of various species, and is both an excellent food and a liquid, thereby offering a maximum chance for diffusion, it would seem that the natural interrelations of associative development would be as evident in milk as anywhere. Thus, with the purpose of studying the possibilities of associative growth in the lactic acid fermentation in milk, a typical strain of Strept. lacticus, isolated from the local dairy starter, and B. subtilis, obtained from the American Museum of Natural History, were grown together.

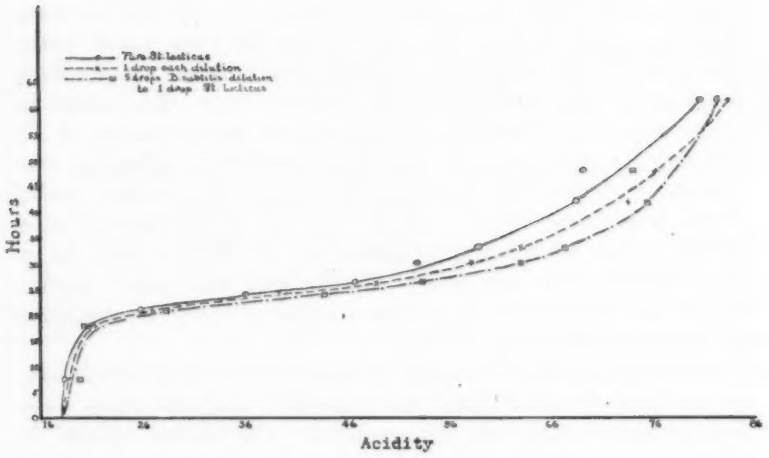
The general procedure apart from variations made for especial details was simple. Milk cultures of the two organisms from twenty-four to forty-eight hours old were used. Dilutions were made from these from which 100 cc. lots of sterile litmus milk were inoculated with varying quantities of the two organisms as desired, using as a unit of the dilution a drop from a Roux pipette. The flasks were kept at room temperature and the developments watched by means of the reduction and oxidation of the litmus and by means of titrations with phenolphthalein. The interest was, of course, focused on the variation in lactic fermentation in associative cultures as contrasted with controls of the pure lactic streptococci.

It was first evident that the presence of B. subtilis in milk did stimulate the lactic fermentation (graph 1). It will be seen from the graph that at any time during the most rapid lactic fermentation that associative cultures give a higher acidity than the control, and furthermore the variance from the control is proportional to a function of the original concentration of B. subtilis. It may be said that the associative influence can scarcely be looked for as increasing beyond 54°, Fuller's scale, at the latest, since the B. subtilis can not develop beyond this acidity. The relationships represented in these curves have been brought out repeatedly, always with the same results. Hence the presence of B. subtilis in milk is likely to stimulate the lactic fermentation.

Knowing the relative courses of these cultures in fermentation, it was desired to look into the numerical changes which were taking place during the fermentation. The results (graph 2) were the best that could be obtained by the plating method.

TIME	LACTIC	1:1 (DROPS)		5:1 (DROPS)	
		Subtilis	Lactic	Subtilis	Lactic
hrs.					
10.0		82,500		319,000	
16.0	65,500	1,230,000		2,050,000	
20.0	406,000	3,270,000	290,000	2,030,000	380,000
24.0		3,180,000		12,720,000	
28.2	11,000,000	19,000,000	170,000,000	12,000,000	122,000,000
45.0	126,000,000	11,000,000	161,000,000	8,270,000	19,080,000
49.2	343,000,000	None	980,000,000	None	1,601,000,000

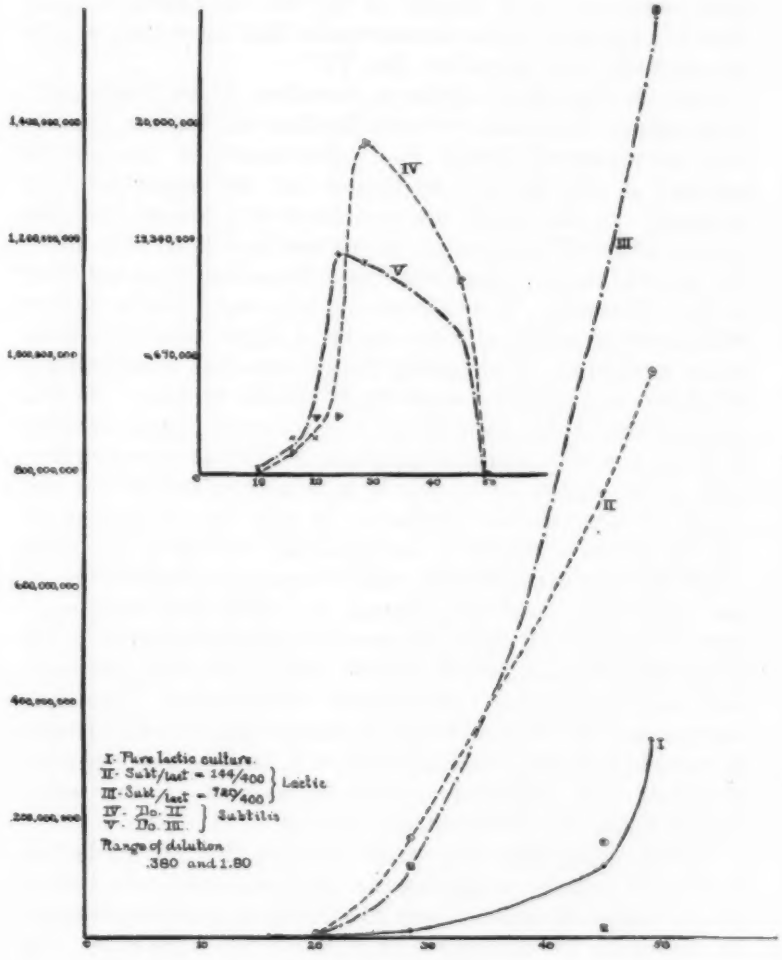
Counting these became a difficult process since both organisms were present of necessity in the same plate. It will be observed that at fifty hours, however, there were no more organisms of B. subtilis so that the last points on the streptococcus curves should be fairly accurate. There is a numerical rise in the Strept. lacticus coinciding with the fermentation, as could only be expected. The B. subtilis shows a rapidly increasing rise up to a certain point when the lactic fermentation gains the ascendancy. It is important to note that the culture receiving originally the



Relative Rate of Rise in Acidity
of Strept. lacticus as associated with B. subtilis.

Hours	Acidity		
	Lactic	1:1	5:1
0	18.0	18.0	18.0
7.5	18.3	18.6	19.6
18.0	20.7	21.3	20.3
21.0	25.7	26.7	28.3
24.0	36.0	39.3	43.7
26.2	46.7	48.7	53.3
30.0	52.7	58.3	63.0
33.0	58.7	63.0	67.3
42.0	68.3	73.3	75.3
48.0	69.0	76.0	73.7
62.0	80.2	83.0	82.0

Graph No. 1



Numerical Rise of Normal and Associative Cultures.

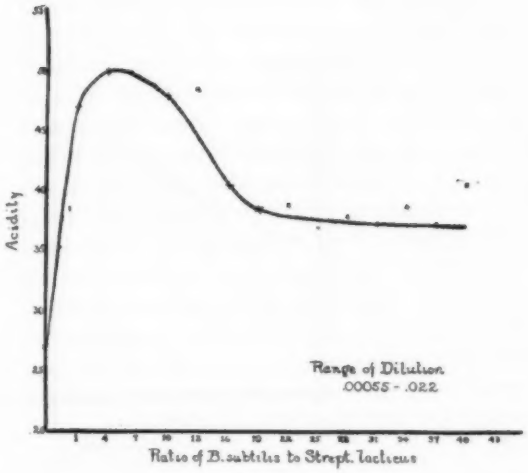
Graph No. 2.

fewer organisms of B. subtilis of the two associative cultures (line IV) reaches a higher concentration than the culture receiving originally more organisms (line V).

There is theoretical evidence, therefore, of an "optimum" initial ratio of B. subtilis to Strept. lacticus, and this has actually been demonstrated (graph 3). Unfortunately it can not be repeated at will, for only by chance can the proper ratio be obtained. In this graph are considered at a certain time the various stages of fermentation in cultures inoculated with different ratios of the two organisms, using the acidity as an indicator to the differences. It is obvious on this curve that a culture with a certain initial ratio has reached a higher stage of fermentation at the time of comparing the cultures than other cultures which received slightly more of the B. subtilis dilution.[1] Several explanations of this are possible, but the most logical seems to be from a mathematical comparison of the curves. A higher ratio of B. subtilis to Strept. lacticus may stimulate the first stages of fermentation sufficiently to stop the increase of B. subtilis before it reaches a concentration reached in a culture which had originally fewer B. subtilis organisms, since the latter has more time to develop (graph 2). Thus the "optimum" ratio would be that which influenced the fermentation up to the point beyond which the B. subtilis will not increase the least, and yet there attained the highest concentration. This concentration would be higher than in those cultures receiving more B. subtilis inoculum, and should hence in the long run give more stimulus. The "optimum" ratio represented in this curve (graph 3) was: B. subtilis/Strept. lacticus = 22/10,000.

Finally comparisons were made with clarified and unclarified associative cultures, presuming the larger organisms to be thrown out more than the smaller, and thus influencing the fermentation by disturbing the associative ratio of the two organisms. The

[1] It is necessary to keep in mind that the rate of fermentation can be stimulated only between the ordinary rate of multiplication of Strept. lacticus and its maximum physiological rate. Hence at room and lower temperatures under the optimum temperature for Strept. lacticus it is capable of greater stimulation than when warm. This has been demonstrated experimentally.



Simultaneous Ratio Effects at Two Different Stages

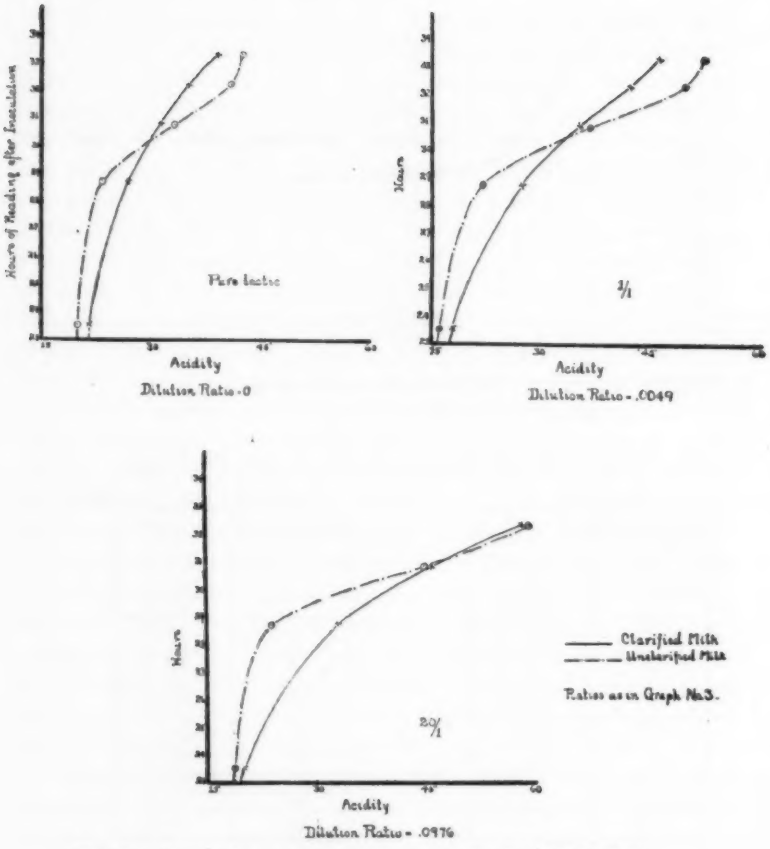
Table No. I

Ratio s/l	Acidity
Lact.	20.0
1	27.5
4	25.0
7	26.0
10	24.5
13	24.0
16	24.0
19	23.0
22	23.0
25	24.0
28	22.0
31	23.0
34	22.5
37	23.5
40	23.0

Table No. II (plotted)

Ratio s/l	Acidity
Lact.	27.0
1	47.0
4	50.0
7	45.5
10	45.5
13	46.0
16	40.5
19	38.5
22	39.0
25	37.0
28	38.0
31	37.5
34	39.0
37	37.5
40	41.0

Acidity or ordinate readings of curve correspond to acidity readings in table.
Dilution drops corresponding to s in ratio of tables furnish the abscissa readings.
The points along curve represent the acidity of the different cultures read at a given moment. Graph No. 3.



Showing Comparison of Clarified and Unclarified Milk in Associative Cultures.

Graph No. 4

results allow of considerable speculation, because so many influences other than association are present and intimately bound together with the introduction of clarification that quantitative conclusions on the associative basis are unwarranted. All that may be safely said is that clarification stimulates lactic fermentation in its early stages, be it with pure or associative culture, sufficiently so that whatever unbalancing of the ratio of the two organisms may take place makes no great apparent difference. Since the clarified cultures in the later stages of fermentation are behind the unclarified in the pure control of Strept. lacticus we can not draw any warranted conclusions from the associative cultures showing the same thing. That the associative influence is present in clarified milk is clear; if clarification influences association, however, its connection with these studies of the two organisms is not apparent.

It may be concluded that: (1) the presence of B. subtilis in milk influences lactic fermentation; (2) the higher the concentration of B. subtilis in the original milk the greater the stimulus in the earlier stages of fermentation; (3) the "optimum" ratio of B. subtilis to Strept. lacticus in the original milk which will give the greater stimulus in the later stages of fermentation is very small—approximately 22 to 10,000; (4) the stimulus is more evident at low temperatures than at high; (5) association plays an undeterminable part in the differences in lactic fermentation caused by clarification.

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THE USE OF FERMENTED MILK AND MILK DIETS IN CONTROLLING INTESTINAL PUTREFACTION

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The use of fermented milks and milk diets in controlling intestinal disorders has long been recognized. Especially the use of buttermilk and milk soured by Bacillus bulgaricus has been recommended by physicians. Earlier investigators have generally ascribed the beneficial action of these sour milks to the antagonistic action of Bacillus lacticus and Bacillus bulgaricus. The lactic acid developed by these organisms was considered as the controlling factor in restraining the development of intestinal putrefactive organisms.

According to Metchnikoff (1) Bacillus bulgaricus can be implanted in the intestine and has the power to destroy harmful bacteria, such as cause putrefaction of the intestinal contents.

Leava (2) a German investigator, carried on extensive experiments upon himself. He also claims that Bacillus bulgaricus can be implanted in the intestine by ingesting large quantities of the organism grown in milk. Bacillus bulgaricus, according to his report, was recovered from the feces after the fifth day, and at the same time the amounts of aromatic oxy-acids, and hippuric acid and conjugated acids in the urine decreased considerably.

Belonovsky (3) experimented upon mice, feeding them sterilized wheat and cultures of Bacillus bulgaricus. After twelve days the character of the intestinal flora showed a marked change from Gram-negative to Gram-positive organisms and a decrease in gas producers. Examination of the feces however failed to show any predominance of Bacillus bulgaricus. He believes that the beneficial action is not due altogether to the Bacillus bulgaricus or the lactic acid, but that other products play an important part also.

Cohendy (4) carried on extensive experiments on thirty hospital patients with the use of milk soured by Bacillus bulgaricus. His results also show a marked change in the character of the intestinal flora. This change was accomplished by the deodorization of the feces and a decrease in the quantities of conjugate sulphates in the urine. His conclusion is that the beneficial results are due to the Bacillus bulgaricus.

Sinclair (5) experimented with milk soured by Bacillus bulgaricus on over a hundred children suffering from infant enteritis and ileocolitis. He also reported a rapid improvement in practically all cases.

Hundreds of other cases could be mentioned proving the value of milk soured by Bacillus bulgaricus in the treatment of intestinal disorders. As stated before, earlier investigators generally ascribed this property to the Bacillus bulgaricus. Recent experimentors, however, seriously question this as the true explanation and attach much more importance to the influence of the diet.

Dr. Rettger (6) of Yale University conducted extensive experiments with young chicks. As in previous cases mentioned, the favorable influence of the milk feeding was very striking. Mortality was frequently reduced to at least one half of that amongst chicks receiving no milk. Milk-fed chicks frequently gained twice as much in weight as those not receiving milk. Chicks artificially infected with Bacterium pullorum (bacteria causing white diarrhea in chicks) showed a much greater resistance when milk fed.

However, the same results were obtained whether Bulgaricus milk or whole sweet milk was fed. His conclusions therefore were that the unique properties of this food exist in milk as such rather than in milk acids or sour milk bacteria that may be present.

Dr. Herter (7) has shown that the products of intestinal decomposition in normal nurslings and bottle-fed babies are remarkably small in amount, when the large number of bacteria inhabiting the lower part of the intestinal tract is considered. He also brings out the fact that the intestinal flora in these cases is a very simplified one. Instead of the varied mixed flora

of the adult, only a few types are predominant. In breast-fed babies Bacillus bifidus and Bacillus acidophilus are predominant. In bottle-fed babies there is a slight increase in the Bacillus coli group and a decrease in the bifidus type, while Bacillus acidophilus predominates.

The great abundance of Bacillus bifidus and Bacillus acidophilus in the simple flora of the healthy nursling or bottle-fed baby should be of considerable interest to the investigator. Especially so when compared with the varied mixed flora of the adult in which Bacillus coli commonly predominates. While Bacillus coli itself is considered a normal inhabitant of the adult intestinal tract, we usually find associated with it a host of other organisms the combination of which produces various degrees of intestinal putrefaction and its resulting disorders and diseases.

Bacillus bifidus and Bacillus acidophilus on the other hand show a strong antagonistic action against the coli type of organisms. They are carbohydrate consuming organisms and slow to attack proteins having practically no proteolytic action. Under favorable conditions they rapidly eliminate the Bacillus coli and its allies. Such favorable conditions seemingly exist in the case of nurslings or bottle-fed babies where we have a minimum of intestinal putrefaction, with a simple flora of the acidophilus and bifidus type.

These facts suggested to the writer that a simplified intestinal flora with Bacillus acidophilus and Bacillus bifidus predominating is associated with a minimum degree of intestinal putrefaction. Further that a milk diet of some kind is essential in bringing about this simplified flora.

It was with these ideas in mind that experiments were started to shed more light on this important question and to determine first, whether or not the simplified flora found in nurslings or bottle-fed babies can be brought about in the adult human being and second whether the organisms themselves are the causative factors or whether their abundance is due to the influence of certain diets furnishing a preferential medium for their development.

To study the effect of various milk diets on the intestinal flora and the amounts of decomposition products produced, six individuals placed themselves on certain milk diets for 28 days. The kind of diets used were:

1. Milk inoculated with Bacillus bulgaricus, Bacillus lacticus and Bacillus acidophilus.
2. Normal sweet whole milk of grade A quality.
3. Watery suspensions of Bacillus acidophilus, Bacillus bulgaricus and Bacillus bifidus.

From 2 to 4 quarts of the various milks were consumed and in addition a regular diet of meat, cereals and vegetables. As a natural consequence of consuming from two to four quarts of milk the quantity of the other foods was very materially reduced.

Observations were made on the nature of the intestinal flora and the production of the decomposition products as indicated by tests for phenol, indol, skatol and indican in the feces and urine. The procedure was to isolate the organisms from the feces by preparing a ten per cent suspension of one part of fecal material in nine parts of physiological salt solution. This suspension was then used for further study as to:

1. Plating on aerobic and anaerobic plates.
2. Staining or smears to study staining properties.

The Gram positive stain was found very helpful because the Bacillus coli group is gram negative, while Bacillus bifidus, Bacillus acidophilus and Bacillus bulgaricus are gram positive.

3. Inoculation of fermentation tubes for gas production.
4. Cultural characteristics in milk.
5. Bio-chemical study for decomposition products as phenol, skatol, indol and indican.

Records were kept as to the movement of the bowels and general welfare of the patient as indicated by gain or loss in weight. More detailed description of methods are reported in Bulletin 104 Storrs Experiment Station (8).

EFFECT OF FERMENTED MILK IN THE DIET

Bacillus bulgaricus is a rather common inhabitant of milk and as such appears as long slender overlapping threads. It is Gram positive and with methylene blue frequently shows granules in the cell. Its optimum growing temperature is 100°F. and in milk it forms a homogeneous curd coagulating the milk usually in six to twelve hours. Action on dextrose, lactose and sucrose broth is characteristic by formation of acid without gas.

Whole milk of grade A quality was pasteurized by heating to 145°F. and holding for thirty minutes. Half of the milk was then cooled to 100°F. and inoculated with a pure culture of Bacillus bulgaricus, while the other half was inoculated with a pure culture of Bacillus lacticus at 70°F. After about 18 hours of incubation at the respective temperatures the milk was again put together in a small hand churn and churned for about ten minutes. The milk was then cooled, bottled and stored in a refrigerator till used. The addition of Bacillus lacticus cultures moderates the acidity and takes away the sharp, puckering taste of a pure bulgaricus culture. The fermented milk thus produced had an approximate acidity of 1.10 per cent.

Five out of six completed the experiment, practically all of whom showed an increase in weight and improvement in physical condition. One of the patients was forced to discontinue the fermented milk due to stomach trouble. At the beginning of the experiment two of the patients selected suffered slightly from indigestion, while all suffered more or less from constipation. All reported improvement in the movement of the bowels. Examination of the feces and urine showed a marked decrease in putrefactive decomposition products. The relief from constipation may have been an important factor in causing a decrease in the decomposition products. Table 1 is a summary of the results.

The intestinal flora in all cases changed from a predominance of Gram negative to a flora which showed a large number of Gram positive organisms. Attempts were made to recover the Bacillus bulgaricus from the feces. The results were unsuccess-

ful in practically all cases. Several times Gram positive organisms resembling Bacillus bulgaricus were isolated. These organisms answer the description of Bacillus acidophilus of Moro. Under the microscope they appear as long slender bacilli which

TABLE 1

Effect of fermented milk in the diet

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+−−	++−	++	+	++	−−	165	168
2	+−−	+++	+++	++−	+++	−−	119	124
3	+−−	++−	++	−−	+	−−	133	134
4	+−−	++−	++++	+−−	++++	−−−	141	150
5	+−−	++−	++	+−	+++	+++	130	129½
6	+−−	++−	+++	++−	+	−	154	154

NOTE: Gram negative is indicated by − which shows that the type of organism is of the coli or putrefactive type.

Gram positive is indicated by + which shows that the type of organism is of the acidophilus or beneficial type.

Patient no. 5 discontinued experiment after five days.

TABLE 2

Formation of acid in milk by Bacillus acidophilus

INOCULATION	INITIAL ACIDITY IN MILK	AFTER 24 HOURS	AFTER 48 HOURS	AFTER 72 HOURS
First	0.15	0.43	0.60	0.65
Second	0.17	0.49	0.61	0.69
Third	0.16	0.63	0.80	0.81
Fourth	0.21	0.53	0.77	0.85
Fifth	0.15	0.75	0.87	0.93
Sixth	0.15	0.75	0.97	1.09
Seventh	0.17	0.83	1.01	1.19
Eighth	0.16	0.90	1.34	1.58

NOTE: These results are an average of six different strains. The highest reached an acidity of 1.97 per cent in seventy-two hours.

show a Gram positive stain. Colonies on plain or whey agar are microscopical in size and resemble colonies typical of Bacillus tetani. Action on dextrose, lactose and sucrose broth were characteristic by formation of acid and no gas. They are very

similar to Bacillus bulgaricus, but did not form acid as rapidly nor is the maximum acidity as high. On propagation in milk cultures they may however develop a considerable acidity as brought out in table 2.

The principal point of difference is that Bacillus acidophilus can readily be recovered from the feces while Bacillus bulgaricus cannot.

When grown in milk Bacillus acidophilus forms a smooth homogenous curd which has a pleasing mild acid flavor. As such it makes a very palatable milk drink and seems much better adapted for use in the preparation of a fermented milk than Bacillus bulgaricus. The acidophilus milk produces the characteristic changes of intestinal flora and decomposition products even more strikingly than the bulgaricus milk and Bacillus acidophilus could be isolated from the feces in from five to eight days.

USE OF SWEET WHOLE MILK IN THE DIET

The procedure of the experiment was the same as where fermented milks were used. All tests and observations were made under as nearly identical conditions as was possible to

TABLE 3

Effect of sweet whole milk in the diet

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+−−	++−	++	+−	++	+−	166½	167½
2	+−−	+−−	+++	+−−	+++	++−	121	127½
3	+−−	+++	+++	+++	+−	++	133	133½
4	+−	++−	+++	++−	+++−	+++−	147	146
5	+−−	+++	++	−−	−−	−−	134	139
6	+−−	+−−	++++	+−−	+−	−−	153½	155½

For explanation see note under table 1.

attain. This time, however, sweet whole milk meeting certified requirements was used. Six patients were fed for a period of twenty-eight days on a regular diet of meats, cereals and vegetables in reduced quantities to allow for the additional food furnished by the daily use of 2 to 4 quarts of milk.

The intestinal flora showed the same marked change as when feeding the fermented milks. It took from eight to ten days before the slides showed considerable numbers of Gram positive organisms of the bifidus and acidophilus types. There was a decrease in the decomposition products. However, patients suffering from constipation were not relieved as they were on the bulgaricus diet. Results are shown in table 3.

Clinical symptoms and general health improved and there was a gain in weight with the exception of two patients who reported severe constipation as a result of the milk diet. Others, however, reported a laxative effect.

USE OF BACILLUS ACIDOPHILUS, BACILLUS BULGARICUS AND BACILLUS BIFIDUS WITHOUT MILK IN THE DIET

Pure cultures of Bacillus acidophilus, Bacillus bulgaricus and Bacillus bifidus were grown on dextrose agar. Watery suspensions of the bacilli were then made from these dextrose cultures.

TABLE 4

Effect of watery suspensions of Bacillus bulgaricus, Bacillus acidophilus and Bacillus bifidus

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+−−	++−	++	++	+−	+−	144	144½
2	+−−	+−−	+−	+−	+−	+−	151	153
3	+−−	+−−	++	+−	−−	−−	152	151½
4	+−−	+−−	−−	+−	−−	−−	121	120
5	+−−	++−	−−	−−	+−	+−	134	136
6	+−−	++−	+−	+−	+−	+−	146	148

For explanation of table see note under table 1.

These watery suspensions were mixed with the food and thus ingested. Six patients followed a regular diet of meat, cereals and vegetables, but no milk was used during four weeks of the experiment. All other conditions were the same as when fermented and sweet whole milk was fed.

No appreciable change could be noticed in the amount of decomposition products produced. The smears showed some

Gram positive bacteria, but large numbers of Gram negative organisms were present throughout the experiment. Bacillus acidophilus, Bacillus bulgaricus and Bacillus bifidus supplied to the diet in the form of watery suspensions seemingly affect the intestinal flora only temporarily and then only to a very slight extent. They do not cause any marked decrease in the production of intestinal decomposition products and their implantation without milk in the intestinal tract is very doubtful. The persons on test did not experience any favorable or unfavorable results from the ingestion of the large numbers of the organisms. Results are indicated in table 4.

GENERAL DISCUSSION AND CONCLUSION

One of the most striking facts to be noticed in the series of experiments is the characteristic change in the intestinal flora coupled with decreased intestinal putrefaction whenever milk constituted a large part of the diet. In these cases the intestinal flora changed from the usually Gram negative coli type to one in which Gram positive aciduric Bacillus acidophilus was prominent. In all instances where milk made up the principal part of the diet there was a marked decrease in the decomposition products.

The results from the use of Bacillus bulgaricus milk in connection with the regular diet was very gratifying. Intestinal putrefaction decreased markedly when at least two quart of the bulgaricus milk was used and the other food reduced proportionately. There was a general gain in weight, and those suffering from constipation were very much relieved. Of special interest is the change in the intestinal flora. Notwithstanding the fact that very large numbers of Bacillus bulgaricus were ingested with the milk, only a few could be recovered from the feces. Bacillus bulgaricus is therefore not a natural inhabitant of the human intestinal tract. The very closely related Bacillus acidophilus could be found in large numbers, but only after about eight or ten days use of the bulgaricus milk. When milk was discontinued the Bacillus acidophilus gradually disappeared

and the flora again showed a large number of Gram negative organisms.

The same held true with the whole sweet milk experiment, which gave practically as good results as bulgaricus milk. This fact becomes still more significant when it is considered that Bacillus acidophilus became a common inhabitant of the intestinal tract without the ingestion of the organism itself. This would indicate that Bacillus acidophilus is naturally an intestinal organism, and that its presence or absence is dependent upon the nature of the food ccnsumed. The diet or nature of the food then is the controlling factor. The character of the intestinal flora is dependent on, and alters with the type of food. The ingestion then of large numbers of certain bacteria in tablet or liquid form without the proper diet can be of little permanent value. This fact was brought out by the experiment in which watery suspensions of Bacillus bulgaricus, Bacillus acidophilus and Bacillus bifidus were consumed. The intestinal flora throughout the experiment showed large numbers of Gram negative types, and there was no appreciable decrease in the decomposition products.

The beneficial effect of milk in the successful treatment of various intestinal diseases as typhoid fever has for sometime been known to the medical profession, but not until recently has it been realized that the explanation lay in its ability to change the intestinal flora from the putrefactive type to the Gram-positive aciduric Bacillus acidophilus group, the presence of which is associated with a minimum amount of putrefaction and the general well-being of the individual. Whether fermented milk or fresh sweet milk is preferable depends upon conditions. If the subject is constipated the fermented milk may prove very helpful. On the other hand, some individuals apparently do not tolerate acid milk, and in such cases the sweet milk would be best. Approximately three to four quarts should be used daily, in half pint quantities taken every hour or two. Other foods should be reduced proportionately, and in certain cases it may be advisable to leave them out entirely and increase the milk to 4 or even 6 quarts, depending on the needs of the patient.

There may be conditions when it is advisable to have in the intestine at once large numbers of the Bacillus acidophilus. A fermented milk prepared with Bacillus acidophilus would be admirably suited for such needs. Instead of using the bulgaricus culture an acidophilus culture should be used. This provides the proper diet in the form of milk and large numbers of the desirable organism which is a natural inhabitant of the intestinal tract. The acidophilus milk has a pleasing mild acid flavor and a rich creamy consistency.

It seems to the writer that such a fermented milk offers great possibilities in the treatment of various intestinal disorders. As yet much work remains to be done especially in preparing a commercial product which can readily be manufactured in the average milk plant.

Experiments covering this phase of the work are now under way in the Dairy Laboratories of the Connecticut State College, at Storrs.

So far the work has brought out the importance of the diet in controlling intestinal putrefaction and disorders. It was shown that a change in the food alters the nature of the intestinal flora, and that milk is the one food which can bring about so characteristic and beneficial a change in the intestinal flora from the putrefactive to the acidophilus type. Already the medical profession is using Nature's Own Food, milk, to combat various diseases, and will continue so even more in the future, as the importance of the proper food in prevention and treatment of disease is realized.

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COMPARISON OF THE DECANTATION METHOD WITH OTHER METHODS FOR THE DETERMINATION OF FAT IN BUTTER

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Many methods which may be used in the laboratory for the determination of the percentage of fat in butter have been devised. None of these methods, however, is directly applicable in creamery control analyses, because of the time involved and the skill necessary in their manipulation. Certain of these methods have been modified in such a manner as to decrease the time and the skill required to obtain a determination of sufficient accuracy for creamery work.

An attempt has been made in this work to compare one of the newer of these modified methods, the Kohman[1] decantation method, with the Mojonnier method and with two of the slower laboratory methods, the A. O. A. C. and the Roese-Gottlieb. In the last three of these methods all weighing is done with an analytical balance.

In the decantation method the moisture was determined in a 10-gram sample, using an aluminum cup with a pour-out lip. The cup was about 2 inches inside diameter and 2¼ inches deep. A Torsion moisture balance—style 1700—was used for all weighing done in both the moisture and the fat determinations. Any other type of moisture balance of equal sensibility could be used just as successfully.

After the completion of the moisture determination, about 50 cc. of petroleum ether was poured into the cup and was thoroughly mixed with the dry butter. The fat dissolved in the ether while the curd and salt, which are insoluble, settled to the bottom of the cup. When the sediment had completely separated (usually not more than two or three minutes are necessary), the ether solution of the fat was poured off, care being taken not

[1] E. F. Kohman, J. Ind. Eng. Chem., xi, 36, 1919.

to lose any of the sediment with the ether. About 30 cc. of the solvent was added to the sediment in the cup. The mixture was stirred, allowed to settle, and the solvent was poured off. In each case the solvent should be poured off as completely as is possible without loss of sediment. The cup with its contents was then dried on a water bath or hot plate. The drying may be done directly over a very small non-luminous flame if care is taken to avoid ignition of the remaining traces of petroleum ether. After the ether had completely evaporated, the cup was cooled and the loss in weight due to the extraction of the fat was determined. A set of weights ranging from 0.01 gram to 5 grams is necessary for this weighing. The moisture weights during the fat determination must be left in exactly the same position as they were placed to show the percentage of moisture. The loss in weight of the cup and contents represents the weight of fat in 10 grams of butter. This weight multiplied by 10 gives directly the percentage of fat in the sample.

The percentage of salt may be determined by applying to the contents of the cup after the completion of the fat determination any of the salt tests based upon a 10-gram sample of butter.

The percentage of curd may be found by difference: 100 − (percentage moisture + percentage fat + percentage salt) = percentage curd.

In table 1 duplicate results obtained in the analysis of eight different samples of butter by each of the four methods named above are listed. In no case does the amount of fat determined by the decantation method vary from the determination by any of the other three methods by as much as 0.2 per cent, and in most cases the difference is less than 0.1 per cent.

As a further verification of the accuracy of the decantation method the ether solution of the fat was poured directly from the cup into a weighed Erlenmeyer flask. The ether was evaporated on an electric hot plate and the flask and fat dried to constant weight at 100°C. in an electric oven. The results of these determinations are listed in table 2. The analyses numbered 1 to 8 are the results obtained from the samples correspondingly numbered in table 1. Analyses 9 to 12 are the results

of samples tested only by the decantation method and by the direct weighing of the fat recovered from the decanted solution.

In 15 of the 24 determinations listed the difference between the two methods is less than 0.1 per cent and in none of the other 9 does it reach as much as 0.2 per cent.

TABLE 1

Comparison of the decantation method with the Mojonnier, Roese-Gottlieb, and A. O. A. C. methods

ANALYSIS NUMBER	A. O. A. C.		ROESE-GOTTLIEB	MOJONNIER	DECANTATION	
	Water	Fat	Fat	Fat	Water	Fat
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	14.29	82.35	82.40	82.41	14.2	82.3
	14.32	82.34	82.37	82.40	14.3	82.3
2	14.08	82.46	82.52	82.46	14.0	82.5
	13.93	82.43	82.49	82.42	14.0	82.4
3	14.17	82.17	82.21	82.19	14.2	82.3
	14.20	82.11	82.20	82.19	14.1	82.2
4	14.66	81.90	81.93	81.93	14.5	81.9
	14.53	81.87	81.89	81.90	14.6	81.8
5	13.69	82.23	82.25	82.29	13.8	82.2
	13.54	82.19	82.24	82.24	13.7	82.2
6	14.95	80.45	80.44	80.35	15.1	80.5
	15.07	80.38	80.36	80.41	15.1	80.3
7	13.81	82.05	82.13	82.05	13.9	82.0
	13.87	82.08	82.09	82.06	13.9	82.0
8	14.54	81.20	81.11	81.14	14.5	81.2
	14.74	81.06	81.15	81.17	14.5	81.1

The results given in the tables show very distinctly the accuracy of the decantation method as compared with other methods used in the analysis of butter for fat.

The decantation method as applied to creamery control work in butter making has the following advantages:

1. The method is accurate, giving results which check very closely with the slower laboratory methods.

2. The method is simple and easy to manipulate. No more skill is necessary for its successful use than is required in the usual moisture determination.

TABLE 2

Comparison of the decantation method with the method of direct weighing

ANALYSIS NUMBER	DECANTATION METHOD, FAT	DIRECT WEIGHING, FAT	DIFFERENCE
	per cent	*per cent*	
1	82.3	82.38	0.08
	82.3	82.32	0.02
2	82.5	82.60	0.10
	82.4	82.58	0.07
3	82.3	82.34	0.04
	82.2	82.28	0.08
4	81.9	82.04	0.14
	81.8	81.92	0.12
5	82.2	82.31	0.11
	82.2	82.24	0.04
6	80.5	80.59	0.09
	80.3	80.41	0.11
7	82.0	82.01	0.01
	82.0	82.14	0.14
8	81.2	81.23	0.03
	81.1	81.25	0.15
9	82.7	82.63	−0.07
	82.7	82.68	−0.02
10	80.75	80.94	0.19
	80.8	80.93	0.13
11	81.2	81.21	0.01
	81.2	81.16	−0.04
12	82.3	82.24	−0.06
	82.3	82.32	0.02

3. Not more than fifteen to twenty minutes are required for the determination of both moisture and fat. A few minutes more will permit the determination of salt and curd if desired.

4. The only apparatus and material necessary in addition to that used in the ordinary moisture test is: (a) a set of weights ranging from 0.01 gram to 5 grams; and (b) a supply of petroleum ether with boiling point 30° to 50°C. or 50° to 60°C. (The expensive fat-free petroleum ether is not necessary.)

ABSTRACTS AND REVIEWS OF DAIRY LITERATURE

MICROBIOLOGY AND MICROANALYSIS OF FOODS[1]

"This volume is intended as a guide to the study of the microbiological decomposition changes in foods. It also presents a practical working basis for ascertaining the decomposition limits of foods suitable for human consumption, by means of the direct methods of microanalysis."

The text of 260 pages is addressed to army dietitians and food examiners and in keeping with the limitations surrounding war conditions attention is directed almost exclusively toward information obtainable through the microscope. To those accustomed to elaborate laboratory equipment the character and variety of the information so obtainable is quite a revelation. Although we are no longer at war there is still need of methods for a rapid and accurate examination of foods and in this volume, which covers practically all kinds of foods, such methods are well arranged and well indexed.

While the question of methods has been well handled there will undoubtedly be much difference of opinion regarding the deductions drawn from examinations of food. His statement that "Grade 'A'and Grade 'B' milks are of equal value to the army" will shock some people. His conclusion that cheeses should not be used as an army food because of the liability of their undergoing secondary toxic decomposition will likewise appear strange to others. His evident suspicion of canned goods is in sharp contrast with the results of the study by Rosenau and his associates.

As a matter of fact, wholesomeness of foods has long been a favorite field for fable and fancy. It is asking too much that the author should divest himself of all the myths of his childhood and confine himself to prosaic facts. Filth, rotting bacteria, and ptomaine poisonings are very real imps to the author and to a majority of the public.

While at present it is frankly impossible to harmonize the extreme views held by many regarding the relations of germ life to wholesomeness of food this author should be credited with an evident desire to take a moderately conservative position in such matters. The book is a valuable contribution and will repay careful examination by those interested in the subject.

H. A. HARDING.

[1] By A. Schneider,
P. Blakeston's Son & Co.

DAIRY NOTES

J. W. HENDRICKSON

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ARKANSAS

Prof. H. E. Dvoradhek of the dairy department, University of Arkansas, reports some investigations in progress in the use of legume crops as silage for dairy cattle. Cow peas and soy beans are used in combination with corn and cane. A progress circular or bulletin may be published soon. The Arkansas station is also doing some research work on the cost of raising dairy cattle.

CALIFORNIA

Among the recent publications from the California University Dairy department is a circular on the subject "The Babcock Test for Butterfat in Milk, Cream and Skimmed Milk," by J. C. Marquardt and a bulletin on "The Use of White Fir and Choice Lumber in the Manufacturing of Boxes Used for Shipping and Storing Butter," by Prof. S. D. Turnbow who scored the butter when it was placed in the boxes and will also make examination at frequent intervals. In California there has been a prejudice against the use of boxes made from any other than spruce lumber. It is believed that there are other woods that are cheaper and that will give just as satisfactory results as the more expensive spruce boxes.

Assistant Professor S. D. Turnbow became a member of the staff of the California Dairy Industry during the present year.

Mr. H S. Beard formerly assistant professor of dairy industry has accepted a position with the Northern California Milk Producers Association.

Mr. S. L. Denning also formerly of the California Dairy Industry Department is now with the sales department of the Premier Machinery Company of San Francisco.

CONNECTICUT

Prof. R. C. Fisher of the Connecticut Agricultural College has a new publication on "The Use of Fermented Milk and Milk Diets to Control Intestinal Putrefaction."

On February 1, 1920, Mr. Wm. A. Rhea, B.S., University of Missouri, M.S. from Cornell University, resigned from his position at the Connecticut Agricultural College as extension dairyman and is now with a banking institution at Pierce City, Missouri. The vacancy was filled by the selection of Mr. P. A. Campbell, a graduate from the New Hampshire State University and formerly professor of animal husbandry at the University of Maine. During the last ten years Professor Campbell has been connected with two of the largest breeding establishments in the East, namely Balsam Stock Farm, Dixville Notch, New Hampshire, and Ayrdale Stock Farm, Bangor, Maine.

IDAHO

Mr. H. A. Bendixen, M.S. of the Iowa State College, has accepted the position of assistant professor of dairy husbandry in the University of Idaho to succeed Mr. E. F. Sass. Mr. Bendixen's work will be along the line of milk testing, butter making, cheese making and ice-cream manufacturing—and will have charge of the teaching and research work along these lines.

ILLINOIS

Professors H. A. Harding and M. J. Prucha, of the Illinois Station, have just issued a bulletin No. 228 on the subject "An Epidemic of Ropy Milk," in which they state that ropy milk is due to the growth of an organism resulting in increased viscosity of the milk. While no evidence has been produced to indicate that the healthfulness or the flavor of the milk have been changed, the consuming public does not look with favor upon this class of product. In the epidemics studied, sanitary methods applied to utensils, milk house equipment and to cows and stable surroundings as well, seem necessary in order to effect complete eradication of the trouble.

IOWA

Prof. B. W. Hammer of the Iowa State College has quite recently issued several general and research bulletins, among which are "Bacteriological Results Obtained in Practice with Vat Pasteurization and with One of the Final Package Methods," also, "A Bacteriological Study of the Methods of Pasteurizing and Homogenizing the Ice Cream Mix." The research bulletins are on the subjects "Studies on

Abnormal Evaporated Milk," "Studies on Formation of Gas in Sweetened Condensed Milk," and "The Volatile Acid Production of Starters and of Organisms Isolated from Them."

In first research bulletin mentioned "An abnormal condition in evaporated milk, evident in the original milk as a bitter flavor and abnormal odor, was studied and found to be due to an organism that it is believed has not been previously described and for which the name Bacillus amarus is proposed."

In the second research bulletin the gas formation in sweetened condensed milk was found to be due to a budding organism. Professor Hammer states that "Since the yeast can grow in a saturated solution it is possible that the milk solids play a part in keeping down growth."

In the study of starters, Professor Hammer assisted by Mr. D. E. Bailey came to the conclusion that more than one organism is often present in starters of satisfactory quality. They also state that the high volatile acidity of starters is not due to the action of B. Lactis acidi alone.

Mr. E. W. Renner, instructor in market milk at the Iowa State College is now with the Decatur Ice Cream Company, Decatur, Illinois.

Mr. S. J. Pearse, formerly a Nebraska man with M.S. degree from Iowa State College has taken the place of Mr. Renner.

Mr. J. F. Jarvies, instructor in ice cream making, left to go with the Douglas Ice Cream Company, of Chariton, Iowa. Mr. R. L. Neasham recently with the Flynn Dairy Company of Des Moines has been selected to fill the vacancy.

KANSAS

Prof. N. E. Olsen, formerly assistant professor of dairy husbandry, is now with the Columbia Dairy Products Company at Vancouver, Washington. Mr. F. W. Athenson in charge of advanced registry work has become field man for the Southwest Jersey Breeders' Cattle Club, with headquarters at Kansas City, Missouri.

MARYLAND

The following bulletins have just been issued by the Maryland Station: "Cooling of Milk and Cream on the Farm," Farmers' Bulletin No. 976, U. S. Dept. of Agri., J. A. Gamble; "The Straining of Milk on the Farm," by Ernest Kelly and J. A. Gamble, Farmers' Bulletin No. 1019; U. S. Dept. of Agri. Bulletin No. 744, "Cooling Milk and

Storing and Shipping It at Low Temperature," by J. A. Gamble and John T. Bowen, U. S. Dairy Division. A list of the breeders of pure-bred dairy cattle in Maryland (in process of publication) by Messrs. Meade and Gamble. The Babcock Testing of Milk by J. A. Gamble.

In coöperation with the Market Milk Section of the United States Dairy Division, a study on the effects of feeds on the flavors and odors of milk is being made.

E. H. Parfitt, graduate student in dairying, has left to take up work in the laboratories of the United States Dairy Division. A graduate assistant to take his place will be available July 1 and a teaching assistant in dairy husbandry, October 1.

R. H. Ruffner, formerly professor of animal and dairy husbandry at this institution, is now head of the animal and dairy husbandry department at the North Carolina A. & A. College, West Raleigh, North Carolina. Mr. A. C. Stanton, formerly his assistant, is in charge of the Seward Stock Farm, Petersburg, Virginia.

MINNESOTA

R. M. Washburn, who was for seven years professor of dairy husbandry in charge of dairy products in this institution, is now with the International Dry Milk Company, of Minneapolis, as technical expert. J. C. Cort resigned his University position during the year to assume the duties as manager of the Milford Meadows Stock Farm, Lake Mills, Wisconsin. E. O. Hansen, formerly assistant professor in this division, has severed his connection with this institution in order to become assistant manager of the Creamery Package Manufacturing Company, Minneapolis.

MISSISSIPPI

A publication on "Testing Milk and Its Products" has just been issued.

Mr. Earl Brentnall has been employed to do investigational work in dairy production.

MONTANA

Montana Station has issued a bulletin No. 131 on sunflower silage for dairy cattle, which should be of interest to those states that are not able to produce good corn silage.

NEBRASKA

Mr. M. N. Lawritson, who has been in charge of the advanced registry testing work has become the dairy specialist in the extension department. Mr. Lawritson's place has been filled by the selection of Mr. J. W. Boehr, who received his M.S. degree in dairying at the University of Nebraska last year.

NORTH DAKOTA

Professor J. R. Keithley who was formerly head of the department of dairy husbandry at North Dakota Agricultural College has gone to the University of Minnesota to take charge of dairy manufacturing.

OKLAHOMA

The principal experimental projects carried on by the department of dairying includes the study of the grading of sour cream for the manufacture of butter during the hot summer months; factors in the commercial manufacture of ice cream which includes a study of the use of extra milk solids for improving the quality and increasing the overrun of ice cream; the commercial manufacture of cheddar cheese and the commercial pasteurization of milk in the final container.

Prof. C. A. Burns, assistant dairy-man, resigned in March to go into commercial work and the vacancy has been filled by the appointment of A. D. Burke of Columbus, Ohio, who comes to the department on July 1, 1920.

PENNSYLVANIA

W. B. Combs, graduate at the University of Missouri, 1915, was placed in charge of the dairy manufacturing work in the Department of Dairy Husbandry, January 1, 1920, in the place of H. C. Yerger, Jr., who resigned to take up commercial work.

SOUTH CAROLINA

South Carolina Dairy Division has the following experimental work under way: (a) Study to determine the most economical concentrate to supplement cotton seed meal as a feed for dairy cows in the south. This experiment is being undertaken in coöperation with the North Carolina and Alabama Stations. Various feeds are being compared with

velvet bean meal and wheat bran. (b) Comparison of corn silage and sorghum silage for milk production. (c) To ascertain the amount of feed necessary and cost of raising dairy calves to two years of age under South Carolina conditions.

SOUTH DAKOTA

Mr. H. A. Mathiesen who has served since September 1919 as graduate assistant in the dairy department of South Dakota State College resigned in March to become assistant field dairyman in Idaho.

CHANGES IN UNITED STATES DAIRY DIVISION PERSONNEL

W. E. Wintermeyer, assistant dairy manufacturing specialist, has resigned to accept a position with the Fisk Tire Rubber Company, at Raleigh, North Carolina.

Geo. J. Miller, dairy manufacturing specialist, engaged in creamery extension work in Mississippi, has resigned to accept a position as manager for the Watson and Avon Ice Cream Company, at Helena, Arkansas.

F. L. Brown, coöperative dairy extension man in New Hampshire, has resigned to become a county agent in New Jersey.

H. J. Childress, coöperative dairy extension worker in Oklahoma, has resigned to accept a position as county agent in the State of Kentucky.

Dr. N. R. Blatherwick, who has been engaged on problems in connection with the physiology of milk secretion, has resigned to become biochemist in charge of the chemical work with the Memorial Laboratory and Clinic, Santa Barbara, California, where he will conduct research work in metabolic diseases.

W. D. Wood, engaged in experiments to determine the food requirements for milk production in Louisiana, has resigned to accept a dairy extension position in South Carolina.

Clarence V. Castle, a graduate of the University of California and an instructor in animal husbandry and superintendent of the farm dairy at that institution for two years, has been appointed for the organization of cow-testing associations in the Western United States with headquarters at Salt Lake City, Utah.

Elliot H. Parfitt, of New York, has been appointed scientific assistant in dairying and assigned to cheese manufacturing investigations in the dairy research laboratories, taking the place of A. C. Weimar who was transferred to Grove City, Pennsylvania. Mr. Parfitt received his

degree of Associate in Agriculture from the Ontario Agricultural College (Guelph), 1916, and his B.S. in Agriculture from Toronto University in 1918. After graduating he was appointed as lecturer in dairy husbandry at Ontario Agricultural College, but enlisted, November, 1918, in the Medical Corps of the United States Army. In the fall of 1919 he was appointed instructor in dairy manufacturing at the Maryland Agricultural College.

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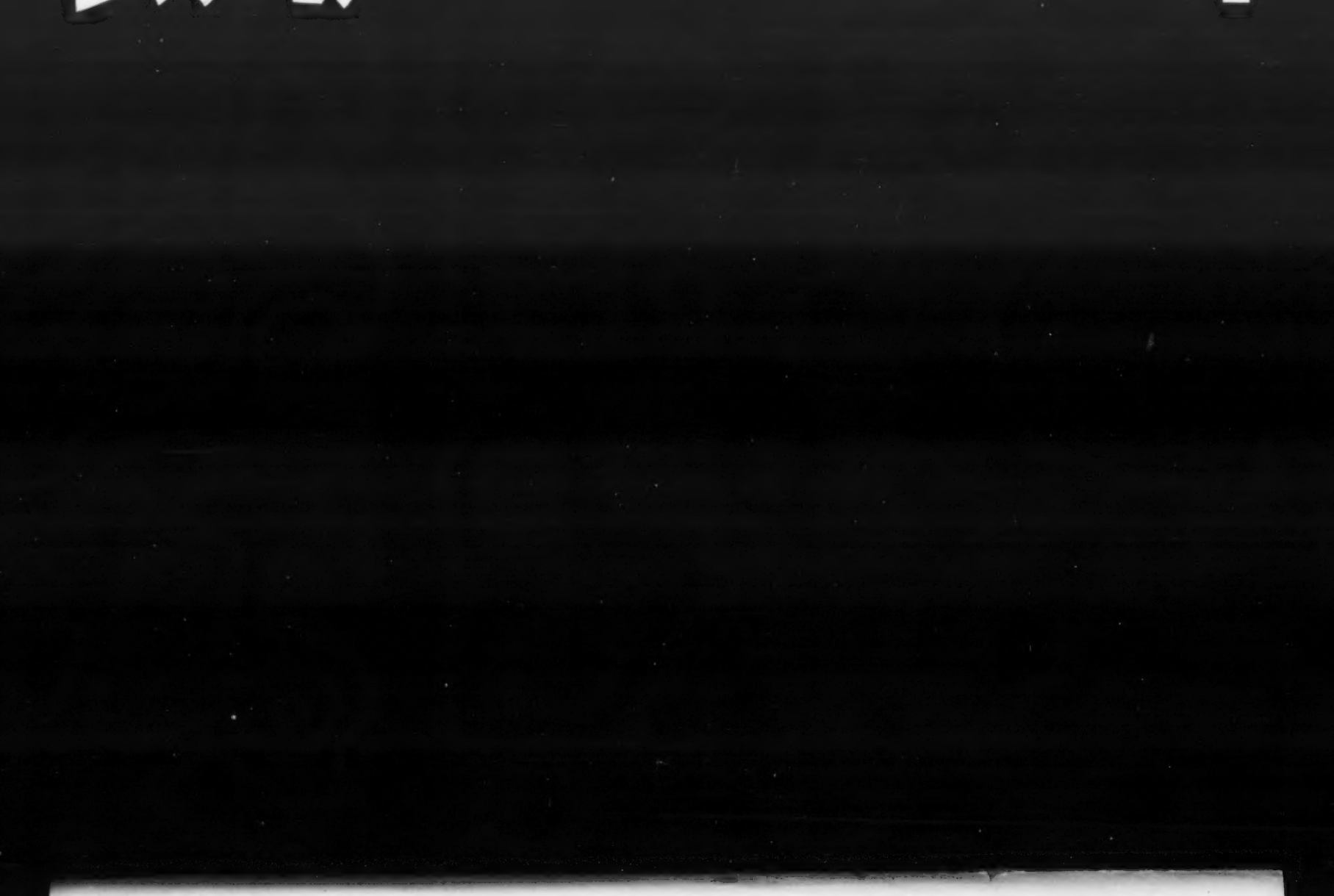

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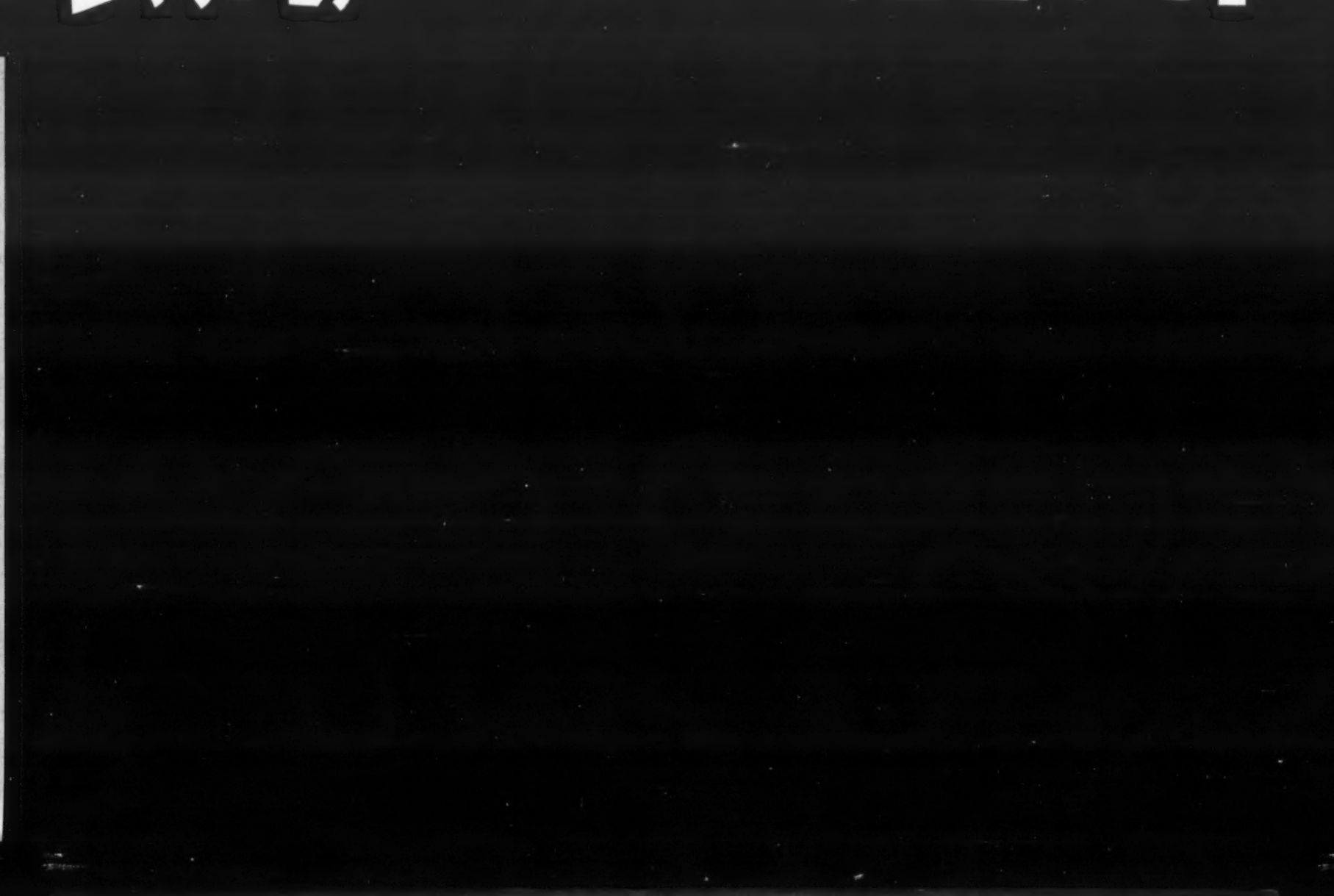
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